REMARKS

Claim Amendments

Claims 1, 2, 26 and 27 are amended to recite "pharmaceutically acceptable" salt.

Claims 1, 2 and 24-28 are amended to specifically recite the transplanted organ or transplanted tissue as "heart, kidney, lung, liver, pancreas, pancreatic islets, brain tissue, stomach, large intestine, small intestine, cornea, skin, trachea, muscle or bladder, or part of heart, kidney, lung, liver, pancreas, pancreatic islets, brain tissue, stomach, large intestine, small intestine, cornea, skin, trachea, muscle or bladder." Support for these amendments can be found, for example, in the 3rd paragraph (lines 16-21) on page 11 of the originally filed application.

Claim Rejection under 35 U.S.C § 102 (a)

The Examiner maintains the rejection of Claims 1-26 under 35 USC § 102(a) and additionally rejects newly presented Claims 27 and 28 under the same section. With respect to Claims 1-26, the Examiner continues to reject the claims under 35 USC § 102(a) as being anticipated by Sneddon *et al.*, WO 01/87849.

Claims 1-28

The Examiner states that "GVHD [Graft-versus-Host Disease] is a form of transplant [rejection]" (see p. 3, third paragraph) and that "[t]he claims as read are not distinguishable from graft versus host disease" (see p. 2, last paragraph).

Applicant maintains to respectfully disagree with this assertion and emphasize that the Examiner does not provide any support for her assertion that Graft-versus-Host Disease is a form of transplant rejection. The Examiner continuously failed to provide any reasoning as to why evidence previously presented to the contrary is not persuasive. The previously presented evidence included three textbook references that teach that Graft Versus Host Disease and graft (transplant) rejection are two entirely distinct and separate conditions. More specifically, 1) GVHD is caused by the graft reacting against the patient, 2) graft (or transplant) rejection is caused by cells of the patient, and 3) GVHD is not understood in the art to be encompassed by the term graft (transplant) rejection and *vice versa*.

Further, the Examiner's reasoning is contradictory; specifically, the Examiner states that in "GVHD the bone marrow rejects the host" (see page 6, lines 3-4). Thus, the Examiner states that GVHD is a form of "host rejection" in contrast to her assertion that GVHD is a form of transplant rejection as discussed above. Applicant's claims, as currently amended, recite inhibition of rejection of a transplanted organ or transplanted tissue, and not inhibition of rejection of the host as implied by the Examiner.

Further support for Applicant's position that GVHD and graft rejection are different conditions is provided by Jamieson *et al.* cited by the Examiner to support the rejection. Jamieson *et al.* teach that a transplant recipient (host) experiencing GVHD is unable to reject the transplant (graft) (see page 67, left-hand side):

> Billingham defined the **requirements of GVHD** as follows [3]:
> 1. histocompatibility differences between donor and recipient;
> 2. presence of immunocompetent cells in the graft; and
> 3. **inability of the host to reject the graft**. (*emphasis added*)

Thus, even if Sneddon *et al.* teach that patients experiencing GVHD can be treated with the instant compounds, they do **not** teach treating patients experiencing transplant rejection, irrespective of the type of transplant, because a patient with GVHD is unable to reject the transplant.

In conclusion, Applicant respectfully requests that the Examiner provides 1) proper support for her assertion that GVHD is a form of transplant rejection and 2) an explanation why Applicant's textbook evidence is considered unpersuasive.

The Examiner also asserts that "[i]nhibiting rejection of a transplanted organ, tissue or cell would be inherent, regardless of the mechanism" (see page 3, last paragraph). Although, Applicant disagrees with these conclusions, Applicant herewith amends Claims 1-28 to expedite prosecution. Specifically, Claims 1-28, as currently amended, recite the transplanted organ or transplanted tissue as "heart, kidney, lung, liver, pancreas, pancreatic islets, brain tissue, stomach, large intestine, small intestine, cornea, skin, trachea, muscle or bladder, or part of heart, kidney, lung, liver, pancreas, pancreatic islets, brain tissue, stomach, large intestine, small intestine, cornea, skin, trachea, muscle or bladder."

It is emphasized that the list of transplanted organs and tissues does not include bone marrow, the principal type of transplanted tissue where GVHD occurs.

Further regarding the Examiner's assertion that "[i]nhibiting rejection of a transplanted organ, tissue or cell would be inherent, regardless of the mechanism", Applicant notes that Jamieson *et al.* cited by the Examiner implies that the risk of developing GVHD after solid organ transplantation is quite small, although actual numbers are not given. A better teaching of the incidence of GVHD after solid-organ transplantation is provided by Gulbahce *et al.* (Am. J. Clin. Pathol. 119(4):568-573, 2003; herewith submitted as Exhibit G). Gulbahce *et al.* searched the surgical pathology files of the University of Minnesota Hospital, Minneapolis, from January 1991 through December 1999 for cases of GVHD in non-bone marrow transplant recipients and found that 10 cases of GVHD developed after 2663 solid-organ transplantations (see page 2):

> All cases of non-bone marrow transplantation-related GVHD occurred exclusively in solid organ transplant recipients. No GVHD was detected as a complication of blood product transfusion alone in patients who had not received a transplant. Ten cases of GVHD occurring in non-bone marrow transplantation recipients were identified. There were 7 men and 3 women (mean age, 45 years; range, 30-58 years). **Five patients received a kidney, 2 received both pancreas and kidney, and 1 each received a liver, lung, or heart (Table 1). During the same period 1,650 kidney, 346 pancreas and kidney, 251 liver, 229 lung, and 187 heart transplants were done in our institution.**(*emphasis added*)

Ten cases of GVHD after 2663 solid-organ transplantations corresponds to an incidence of less than 0.4%. It is emphasized that this small incidence includes a case of GVHD after liver transplantation, and that Assi *et al.* state that "[t]he scientific literature suggests that GVHD following solid organ transplantation is most commonly encountered in liver transplant recipients ..." (Clin Transplant 2007, Vol. 21, pages 1-6; herewith provided as Exhibit H; see first paragraph) How rare and little known GVHD after non-hepatic solid organ transplantation is, is further taught by Assi *et al.* (see page 2, first paragraph of "Literature Review" section):

> A Medline search was conducted to identify **reported cases of GVHD in solid organ transplantation from January 1, 1960 to November 1, 2005** using the terms "GVHD" and "organ transplant." A secondary search was performed to include references cited in the articles obtained on the initial search. Including the patient we are reporting herein, there was a total of **30 cases of GVHD** occurring in recipients of solid organs other than a solitary liver allograft (2–9). (emphasis added)

To emphasize, only reported 30 cases of GVHD in a 45 year period were found in the Medline search. In striking contrast, GVHD after bone marrow transplantation is well recognized as, for example, stated by Jamieson *et al.* in the abstract. This is not surprising considering that GVHD strikes some 30-60 percent of bone marrow transplant recipients as taught by A. Gonski of Children's Hospital Boston (http://www.childrenshospital.org/ newsroom/Site1339/mainpageS1339P1 sublevel268.html; herewith provided as Exhibit I), which corresponds, for example, for the year 2005 alone, to about 3000 to 6000 cases of GVHD considering that about 10000 bone marrow transplantations were performed world-wide in 2005 as provided by Rood and Oudshoorn (Bone Marrow Transplantation (2008), Vol. 41, pages 1-9; herewith provided as Exhibit J; see Figure 2)

The Examiner is reminded of the applicable law of inherency as stated in Section 2112 IV of the Manual of Patent Examining Procedures:

> The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic.
>
> ...
>
> "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.' "
>
> ...
>
> "[a] prior art reference that discloses a genus still does not inherently disclose all species within that broad category" but must be examined to see if a disclosure of the claimed species has been made or whether the prior art reference merely invites further experimentation to find the species.

> "In relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic **necessarily flows** from the teachings of the applied prior art." (*emphasis added*)

Applicant respectfully submits that inhibiting rejection of a transplanted organ or transplanted tissue as recited in the currently amended claims does not **necessarily flow** from the teachings of Sneddon *et al.*, because 1) GVHD is common after bone marrow transplantation but has a low incidence after solid organ transplantation according to Gulbahce *et al.* and 2) the claims, as currently amended, do not include bone marrow as possible transplant.

It is further emphasized that Sneddon *et al.* neither disclose a case of treating GVHD nor a case of treating transplant rejection.

In conclusion, Applicant respectfully submits Claims 1-28 are not anticipated by Sneddon *et al.* for the reasons given above. Withdrawal of the instant rejection is respectfully requested.

Claims 2-23, 25, 27 and 28

In the previous response, Applicant presented amended Claims 2-23 and 25 and new Claims 27 and 28 directed to methods that are directed to inhibiting chronic rejection of a transplanted organ or transplanted tissue in a subject in need thereof. Applicant has carefully reviewed the Examiner's response on page 4 of the Office Action which the Examiner asserts to refer to the term "chronic Rejection" and studied the NIH call for grant applications newly provided by the Examiner. However, Applicant finds the Examiner's argument flawed and incomprehensible for the following reasons. Specifically, the Examiner states

> **As to the terms "chronic Rejection" As evidence by The National Institute of health describes** Transplantation of bone marrow and solid organs such as kidney, heart, liver and lung remains the treatment of choice for several disease states. Although recent progress has improved the short-term survival of allografts, immunological rejection is still an impediment to long-term survival. Substantial evidence has been accumulated indicating that matching the donor organ and the patient for major histocompatibility complex (MHC) antigens can improve both solid organ and bone marrow graft survival. However little is

> known about the extent to which minor histocompatibility antigen (MiHA) mis-matches affect graft survival. For example, in patients who have been treated to prevent graft rejection, approximately half of HLA-matched bone marrow recipients develop acute or chronic GVHD and this is believed to result from MiHA mis-matches. Similarly, while short-term solid organ transplant survival is positively affected by MHC matching, long-term graft survival is still poor with only 40% of kidneys surviving more than ten years.
>
> **Applicant's argument have been given considerable weight but found unpersuasive.** (*emphasis added*)

Applicant emphasizes the Examiner's words in bold font and note that the remaining plain text is taken verbatim from the NIH call for grant applications (see first paragraph of "Background" section). Clearly, the Examiner merely intended to refer the reader to the NIH search for some "evidence" in connection with "chronic rejection" in the subsequent text. However, the Examiner seems to have found a causal link between the two diseases recited when the NIH intended none. The text excerpt from the NIH call for grant applications is a setting for the NIH request for *further research* to be conducted to bring light into a *yet unknown* mechanism of action in two *separate* diseases. In this text excerpt, the only teaching that Applicant finds to relate to "chronic rejection" is the following:

> Similarly, while short-term solid organ transplant survival is positively affected by MHC matching, long-term graft survival *is still poor* with only 40% of kidneys surviving more than ten years.

But this teaching strongly supports Applicant's argument made in the previous response that the prevention of chronic rejection is an *unsolved* problem of the prior art. Thus Applicant finds no valid basis for the Examiner's conclusion that "[a]pplicant's argument have been given considerable weight but found unpersuasive."

Thus, Applicant respectfully requests that the Examiner provides a sound and valid explanation why Applicant's evidence is considered unpersuasive, and where in Sneddon *et al.* there is a teaching of treating chronic rejection.

Applicant repeats the arguments regarding "chronic rejection" for the Examiner's consideration:

Chalasani *et al.* (see Journal of Immunology, 2004, 172: 7813-7820; previously submitted as Exhibit D; page 7813, lines 1-14) teach with respect to acute and chronic rejection:

> The host's immune response to donor Ags leads to **two types of allograft rejection that differ histologically and clinically. Acute rejection is characterized by an intense cellular and humoral attack on donor tissue that results in rapid graft loss. Chronic rejection in contrast is a more insiduous process characterized by obliterative vasculopathy and parenchymal fibrosis that lead to progressive graft failure** (1). The risk of acute rejection in humans is highest in the early posttransplantation period, but declines dramatically over the ensuing months. **In contrast, the risk of chronic rejection increases gradually and becomes a significant cause of graft loss after the first year of transplantation.** (*emphasis added*)

Accordingly, chronic rejection is 1) a type of transplant rejection, 2) differs histologically and clinically from acute rejection, and 3) becomes a significant cause of graft loss after the first year of transplantation.

Further, Hutchinson (Biomarkers and Surrogate Endpoints: Advancing Clinical Research and Applications, April 16, 1999; Natcher Conference Center, National Institutes of Health, Bethesda, Maryland; conference abstract available at http://www4.od.nih.gov/biomarkers/b9.htm ; see Exhibit E) teaches:

> Acute cellular rejection is strongly associated with high-producer TNF-alpha genotype in heart and kidney recipients, whereas IL-10 and IFN-gamma play modulating roles. **Chronic rejection (including declining graft function, transplant vasculopathy, graft loss, and patient death) is strongly associated with high-producer TGF-beta 1 genotype**. (*emphasis added*)

Thus, it is suggested that TGF-beta 1 and not TNF-α is the pivotal cytokine in chronic transplant rejection. The Examiner has provided no evidence to show that chronic rejection is a TNF-α mediated condition, whereas there is evidence in the art to at least suggest that other mechanisms may be involved.

Sneddon *et al.* do not teach that the compounds described therein can be used to inhibit acute and chronic rejection of a transplanted organ or transplanted tissue in a patient in need thereof. Thus, for this reason alone, Claims 2-23, 25, 27 and 28 are novel in light of Sneddon *et al.*

In conclusion, for the reasons given above, independently, Claims 1-28 are novel in light of the Sneddon *et al* reference. Accordingly, withdrawal of the rejection is respectfully requested.

Claim Rejection under 35 U.S.C § 103 (a)

Claims 1-28

Claims 1-28 are rejected under 35 USC § 103(a) as being unpatentable over Sneddon *et al.*(WO 01/87849) taken with Sviland *et al.* (J. Clin. Pathology 1999, 52:910-913) in view of Jamieson *et al.* (Transplant Int. 1991, 4:67-71).

Applicant respectfully disagrees with the Examiner's conclusion that the aforementioned prior art references render Claims 1-28 obvious.

None of the references cited by the Examiner teach or suggest administration of the compounds of the instant invention to inhibit rejection (and, in particular, chronic rejection) of a transplanted organ or transplanted tissue in a subject in need thereof, wherein the transplanted organ or transplanted tissue is heart, kidney, lung, liver, pancreas, pancreatic islets, brain tissue, stomach, large intestine, small intestine, cornea, skin, trachea, muscle or bladder, or part of heart, kidney, lung, liver, pancreas, pancreatic islets, brain tissue, stomach, large intestine, small intestine, cornea, skin, trachea, muscle or bladder.

The Sviland *et al.* reference is directed to the prediction of GVHD following bone marrow transplantation based on a human skin explant model (see title). The Examiner explains the relevance of the Sviland *et al.* reference as follows (see page 6 of instant Office Action):

> Sviland et al. teach GVHD is a complication following bone marrow transplantation (see abstract), wherein (tumor necrosis factor-alpha) TNF-α are important mediators of the cellular damage. (see abstract also)

Applicant respectfully submits that this teaching does not contribute to the teaching of Sneddon *et al.*, because it is already known from Sneddon *et al.* that GVHD is a TNF-α mediated condition. There is no teaching in Sviland *et al.* regarding transplant rejection or the compounds of the present invention, and thus Sviland *et al.* does not cure the deficiency of Sneddon *et al.*

The Examiner additionally explains the relevance of the Jamieson *et al.* reference as follows (see page 6 of instant Office Action):

> Jamieson et al. teach that GVHD is a solid organ transplantation effect. The claims recite transplantation of an organ, tissue or cell (see abstract-highlighted sec.). This is well within the claim limitation.

It is again emphasized, that Graft Versus Host Disease (GVHD) and graft (transplant) rejection are two entirely distinct and separate conditions (see detailed discussion in Applicant's previous response filed January 4, 2007 and the discussion above).

Further, Jamieson does not state that "GVHD is a solid organ transplantation effect." Jamieson merely teaches that GVHD may occur after solid organ transplantation and implies that the risk of developing GVHD after solid organ transplantation is quite small, although actual numbers are not given. As detailed above, less than 0.4% of solid-organ transplantations in the patients studied by Gulbahce *et al.* (Exhibit G) lead to GVHD, and as provided by Assi *et al.* (Exhibit H), only 30 cases of GVHD were found in a 45 year period in publications in the Medline database. Thus, in contrast to the Examiners assertion that "GVHD is a solid organ transplantation effect," GVHD is practically unknown in the context of solid organ transplantation.

The Examiner maintains that the motivation to combine Sneddon *et al.*, Sviland *et al.* and Jamieson *et al.* comes from Jamieson *et al.* (see page 6 of previous Office Action). Again, Applicant respectfully disagrees. Although, all of the references include a teaching regarding GVHD, neither one nor a combination of these references provides motivation to combine them in the context of the inhibition of transplant rejection.

Furthermore, the teachings of Sneddon *et al.*, are limited to a method of treating a TNF-α mediated condition, for example, GVHD in a subject, and Jamieson *et al.* teach that GVHD

requires "the inability of the host to reject the graft." As such, Jamieson *et al.* further supports Applicant's position that GVHD and graft rejection are opposite conditions and Jamieson *et al.* therefore provide no reason to believe that a drug suitable for treating one would have any beneficial effect on the other.

Applicant notes that the Sviland *et al.* reference is irrelevant in this context.

Claims 2-23, 25, 27 and 28

Claims 2-23, 25, 27 and 28 are directed to inhibiting chronic rejection of a transplanted organ or transplanted tissue in a subject in need thereof. As discussed above, Chalasani *et al.* teach that acute and chronic rejection exhibit divergent histological and clinical characteristics, for which the mechanisms are not well understood. Thus, independent of TNF-α being involved in the mechanisms of acute and chronic rejection or not, a person having ordinary skill in the art would not expect that the compounds taught by Sneddon *et al.* would treat both acute and chronic rejection. Again, Jamieson et *al.* and Sviland *et al.* do not contribute any teaching relevant in this context.

Furthermore and supporting the above, Goodman and Mohanakumar (Front. Biosci., 2003, Sept. 1;8:838-44; Exhibit F; see abstract) teach that although acute rejection rates have diminished markedly due to development of new generations of immunosuppressants, the challenge to prevent chronic rejection remains unsolved:

> Current strategies for immunosuppression following organ transplantation focus on the prevention of acute rejection. **As new generations of immunosuppressants have been developed, acute rejection rates have diminished markedly. The new challenge, then, is to prevent the devastating complications of chronic rejection, which have remained largely unchanged over the decades.** The process of chronic rejection is a complex one, and it is likely that most, if not all, components of the immune system play some role in the long-term, smoldering failure of organs following transplantation. (*emphasis added*)

Applicant emphasizes that this is supported by the NIH call for grant applications cited by the Examiner as discussed above.

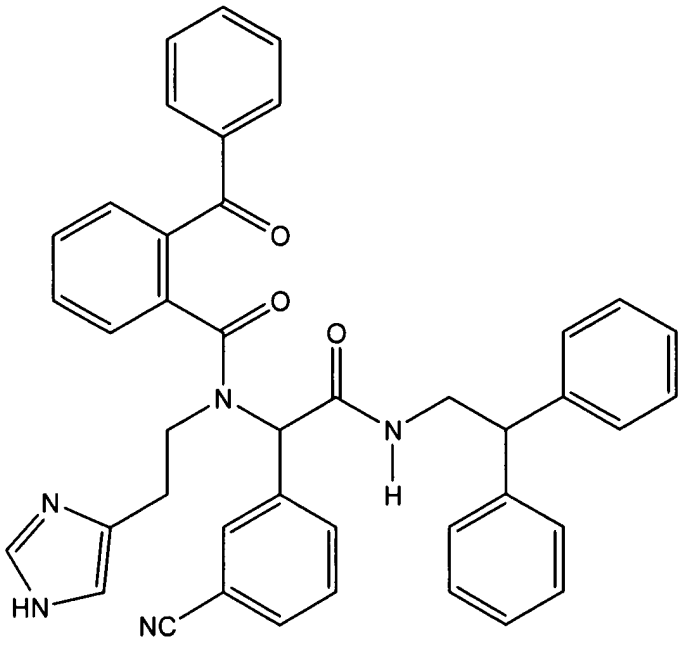
Further, Hutchinson (see Exhibit E) teaches:

> Acute cellular rejection is strongly associated with high-producer TNF-alpha genotype in heart and kidney recipients, whereas IL-10 and IFN-gamma play modulating roles. **Chronic rejection (including declining graft function, transplant vasculopathy, graft loss, and patient death) is strongly associated with high-producer TGF-beta 1 genotype**. (*emphasis added*)

Thus, it is suggested that TGF-beta 1 and not TNF-α is the pivotal cytokine in chronic transplant rejection. The Examiner has provided no evidence to show that chronic rejection is a TNF-α mediated condition, whereas there is evidence in the art to at least suggest that other mechanisms may be involved.

The Applicant has now discovered that the compounds described in the instant specification are effective in preventing chronic transplant rejection. The instant specification page 2 lines 27 to page 3 line 12 states:

> In one example, the histopathological evidence of chronic rejection was inhibited in two mouse models by Compound **1**, shown below. In the first model, chronic rejection of fully MHC class II mismatched transplanted hearts in recipient mice at eight weeks post surgery was inhibited by treatment with 75 mg/kg/day of



Compound **1**

Compound **1** alone during the two weeks following surgery. In the second model, chronic rejection of fully MHC class II mismatched transplanted hearts in recipient mice at 120 days post surgery was inhibited when treatment with 75 mg/kg/day of Compound **1** during the two weeks following surgery was combined with a single administration of 250 ~g of anti-CD154 monoclonal antibody immediately following transplant surgery. Treatment with anti-CD154 monoclonal antibody alone suppresses acute rejection, but is ineffective in preventing chronic rejection of transplanted tissue.

In summation, while Sneddon *et al.*, have a generic teaching of a method of treating a TNF-α mediated condition in a subject there is no specific teaching of treating chronic transplant rejection. As discussed above, chronic transplant rejection is a specific type of transplant rejection, the underlying causes of which are not fully understood and for which there is currently no known treatment. The Applicant has discovered that the compounds described in the instant specification are effective in preventing chronic transplant rejection. Specifically, Applicant has found that the instant compounds inhibit chronic rejection of fully MHC class II mismatched transplanted hearts in recipient mice at eight weeks post surgery. Therefore, the

instant invention represents a surprising and unexpected improvement in methods for inhibiting rejection of tissue transplants that could have not been predicted based on Sneddon *et al.* Again, Jamieson *et al.* and Sviland *et al.* do not contribute any teaching relevant in this context.

In conclusion, for the reasons given above, independently, the Claims 1-28 are non-obvious and patentable in light of Sneddon *et al.*, Sviland *et al.* and Jamieson *et al.*, and withdrawal of the rejection is respectfully requested.

Clarification of Prior Remarks

Applicant wishes to clarify remarks made in the Amendment mailed August 13, 2007 (see page 18, second paragraph) in response to the Office Action mailed by the United States Patent and Trademark Office on April 11, 2007.

The present invention contemplates inhibiting transplant rejection in subjects that are not yet immunosuppressed and also in subjects that are immunosuppressed, for example, because they have received and/or are receiving immunosuppressive drugs. Thus, Applicant's argument should not be construed to mean that these immunosuppressed subjects are not in need of inhibition of transplant rejection. Applicant's argument was merely provided to further support the position that GVHD and transplant (graft) rejection differ.

Provisional Claim Rejection under the judicially created doctrine of Double Patenting

The Examiner maintains her rejection of Claims 1-26 under the judicially created doctrine of double patenting over Claims 1-20 of Application No. 10/719,701 (recently allowed).

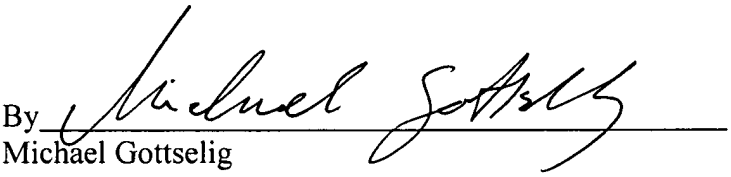
Applicant files concurrently with this amendment a Terminal Disclaimer addressing the Double Patenting Rejection.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By 

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Dated: April 1, 2008

Graft-vs-Host Disease After Solid Organ Transplant

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Am J Clin Pathol 119(4):568-573, 2003.

Posted 04/30/2003

Abstract and Introduction

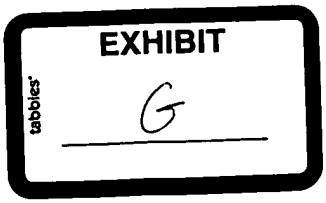
Abstract

We identified 10 solid organ transplant recipients with a histologic diagnosis of graft-vs-host disease (GVHD). Histologic slides were reviewed, and information on the transplant, HLA match, and blood product transfusion history was obtained. Molecular testing to evaluate the presence of donor lymphocytes (chimerism) was done in 1 case. All patients underwent at least 1 gastrointestinal biopsy; 1 patient also underwent a skin biopsy and 1 patient a liver biopsy; all specimens showed grade I to IV acute GVHD. Six patients had a diagnosis of GVHD within 3 months of blood product transfusion and/or solid organ transplantation, which is the time frame in which GVHD reportedly occurs after solid organ transplantation; 4 patients had a distant history of blood product transfusion or solid organ transplantation. In 1 patient, a molecular technique using the polymorphic marker DIS80 documented donor lymphocytes in the colonic tissue and blood (chimerism). Although histologic findings of GVHD are quite specific, they are not pathognomonic. A GVHD-like histologic pattern can be seen in other conditions such as drug reactions and viral infections. Demonstration of donor lymphocytes in the involved organ helps support the diagnosis of GVHD in questionable cases.

Introduction

Graft-vs-host disease (GVHD), a common complication of allogeneic bone marrow transplantation, also may be seen after solid organ transplantation and in immunocompromised people who are given nonirradiated blood products.[1-7] Billingham[8] summarized the donor and host requirements for induction of GVHD as follows: (1) The graft must contain immunologically competent cells. (2) The host must possess important tissue antigens that are lacking in the donor. (3) The recipient must be incapable of mounting an effective immunologic response against the graft cells.

Cellular blood products and solid organs for transplantation contain immunologically competent cells, which puts solid organ transplant recipients at risk of development of GVHD. GVHD after solid organ transplantation occurs 1 to 11 weeks after the transplant and can appear in 2 forms.[2] The more common form is an antibody-mediated reaction in patients who have the A, B, or AB blood type who receive a solid organ transplant from a donor with the O blood type. Antibodies of donor origin directed against the recipient red cell antigens cause mild and transient hemolytic anemia.[9] The second type of GVHD is the cellular type, which primarily affects the skin, gastrointestinal tract, liver, and bone marrow. GVHD is graded clinically on a scale of I to IV based on the severity and number of organ systems involved. A histopathologic grading system also has been established for each of the organ systems involved, but it does not necessarily correlate with clinical severity.[10, 11] We report 10 cases of GVHD diagnosed histologically in solid organ transplant recipients.



Materials and Methods

The surgical pathology files of the University of Minnesota Hospital, Minneapolis, from January 1991 through December 1999 were searched for cases of GVHD in non-bone marrow transplant recipients. Histologic slides were reviewed, and information related to the type of transplant, degree of HLA match, and blood product transfusion was obtained from clinical and laboratory records.

All tissue samples were fixed in buffered formalin and processed routinely. Immunohistochemical staining for cytomegalovirus (CMV) was performed in all cases (monoclonal antibody, 1:20 dilution, DAKO, Carpinteria, CA). Genetic chimerism studies were done as described.[12, 13] For 3 cases (8-10), frozen donor WBCs obtained before transplantation were available. Fresh colonic mucosal tissue was obtained in case 10. For other cases, only formalin-fixed, paraffin-embedded tissue samples (skin, gastrointestinal tract, or both) were available. DNA was extracted from blood and tissue using a modified salt precipitation method (Gentra Systems, Minneapolis, MN). The polymorphic variable number of tandem repeats marker D1S80 was amplified in a 50-μL volume containing 50 to 100 ng of genomic DNA template, a 1.5-mmol/L concentration of magnesium chloride, a 10-mmol/L concentration of tris(hydroxymethyl)-aminomethane, pH 8.8, a 50-mmol/L concentration of potassium chloride, a 1-mmol/L concentration of each deoxynucleoside triphosphate, a 1-μmol/L concentration of each primer, and 2.5 U of *Taq* polymerase (Perkin-Elmer, Shelton, CT). The polymerase chain reaction (PCR) cycling conditions were 22 cycles of 94°C for 1 minute, 67°C for 1 minute, and 70°C for 4 minutes. The PCR primers used were 5'-FAM-GAAACTGGCCTCCAAACACTGCCCGCCG-3' and 5'-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3'. Fluorescent PCR products were analyzed by 6% denaturing polyacrylamide gel electrophoresis on a 373 automated DNA sequencer (Applied Biosystems, Foster City, CA). The generated data were analyzed by using the GeneScan software package (Applied Biosystems).

Results

All cases of non-bone marrow transplantation-related GVHD occurred exclusively in solid organ transplant recipients. No GVHD was detected as a complication of blood product transfusion alone in patients who had not received a transplant. Ten cases of GVHD occurring in non-bone marrow transplantation recipients were identified. There were 7 men and 3 women (mean age, 45 years; range, 30-58 years). Five patients received a kidney, 2 received both pancreas and kidney, and 1 each received a liver, lung, or heart (Table 1). During the same period 1,650 kidney, 346 pancreas and kidney, 251 liver, 229 lung, and 187 heart transplants were done in our institution.

Four patients received organs from living related donors, 4 from cadaver donors only, and 2 from both living related and cadaver donors with 2 to 6 HLA antigen mismatches. All patients received blood product transfusion, none of which was irradiated. For all cases, at least 1 gastrointestinal tract biopsy specimen was available (Table 1). One patient also underwent liver biopsy and 1 skin biopsy. The liver biopsy (case 10) showed damage to interlobular bile ducts characterized by dilatation of the ducts, flattening and vacuolization of the epithelium, and intraepithelial lymphocytes in the absence of portal inflammation. Occasional apoptotic cells and canalicular cholestasis within the lobules were noted. This pattern of injury strongly favors a GVHD-like reaction rather than viral hepatitis or a drug reaction. The mean times between solid organ transplantation and biopsy and the most recent blood product transfusion and biopsy were 469 days (range, 6-2,052 days) and 215 days (range, 1-660 days), respectively.

GVHD developed within 3 months of solid organ transplantation and/or blood product transfusion in 6 cases and more than 3 months afterward in 4 cases. Four patients had mild (grade I) GVHD of the gastrointestinal tract characterized by apoptotic cells and rare intraepithelial lymphocytes in the absence of substantial inflammation in the lamina propria. Four patients had severe GVHD of the gastrointestinal tract with extensive crypt destruction (grade III) or denudation of the mucosa (grade IV) (Figure 1). One patient had grade III GVHD in the skin biopsy specimen, which showed dyskeratotic cells and subepithelial vesicle formation (Figure 2). One patient (case 8) died of disseminated fungal infection complicating marked bone marrow hypoplasia 27 days after the gastrointestinal tract biopsy. The autopsy showed grade III GVHD of the gastrointestinal tract and the skin and markedly hypocellular bone marrow.

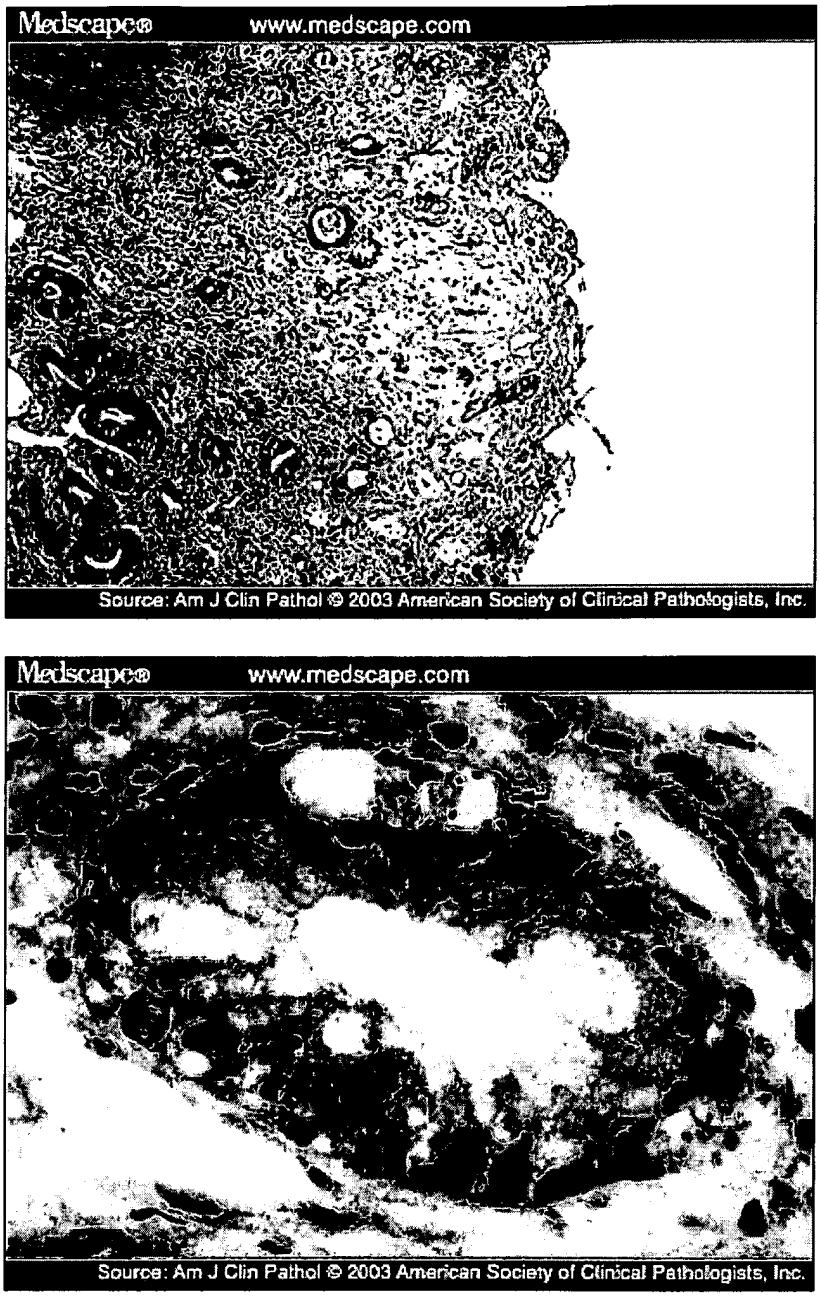


Figure 1. Colonic biopsy specimen from patient 10 shows acute graft-vs-host disease, grade III, with crypt destruction (**A**) and apoptotic cells (**B**, arrow) (A, H&E, x100; B, x400).

Figure 2. Skin biopsy specimen from patient 8 shows acute graft-vs-host disease, grade III, with dyskeratotic cells (arrow-head) and subepithelial vesicle formation (arrow) (H&E, x200).

Immunohistochemical analysis for CMV was negative in 9 cases. One patient (case 10) had CMV inclusions in the colonic biopsy specimen. This patient received pancreas and a kidney from a cadaver and a living related donor, respectively. The kidney graft was removed 1 month after transplantation. Gastrointestinal symptoms started 2 months after transplantation and 1 month after removal of the kidney. Repeated gastrointestinal tract biopsies revealed persistence of the apoptotic cells, crypt destruction, and CMV inclusions. The degree and extent of crypt destruction was disproportionate to the number of CMV inclusions, which prompted evaluation of a possible coexistent GVHD. The pancreas transplant was removed 4 months after transplantation; it showed no viral cytopathic changes on routine H&E stains, but rare CMV inclusions were revealed by immunohistochemical analysis. A liver biopsy on this patient revealed grade I GVHD and no viral inclusions.

Genetic chimerism studies were performed to determine the presence of donor lymphocytes in affected organs and in the blood of 1 patient. Frozen donor WBCs obtained before transplantation were available in 3 cases (8-10). For case 10, DNA was extracted from the posttransplantation blood and colon biopsy specimens and from stored, frozen donor WBCs. DNA from the pancreas donor was heterozygous for the D1S80 marker, with 2 distinct alleles having sizes of approximately 530 base pairs (bp) and 545 bp. DNA from the blood of the patient contained 4 distinct alleles (514, 530, 545, and 595 bp), consistent with chimerism in the blood; alleles of 514 and 595 bp were attributed to the patient. Of the DNA extracted from the colon biopsy specimen, approximately 80% was derived from the patient and 20% from the pancreas donor (Figure 3). Of the DNA extracted from the blood, approximately 94% was derived from the patient and 4% from the pancreas donor. Frozen donor and recipient blood cells also were available for cases 8 and 9. In both cases, the donors and the patients were heterozygous for 2 distinct D1S80 alleles. However, in these and 4 other cases in which paraffin-embedded tissue samples were available, DNA could not be amplified from the tissue to evaluate chimerism. Two of the biopsy specimens were small and were excluded from the molecular studies.

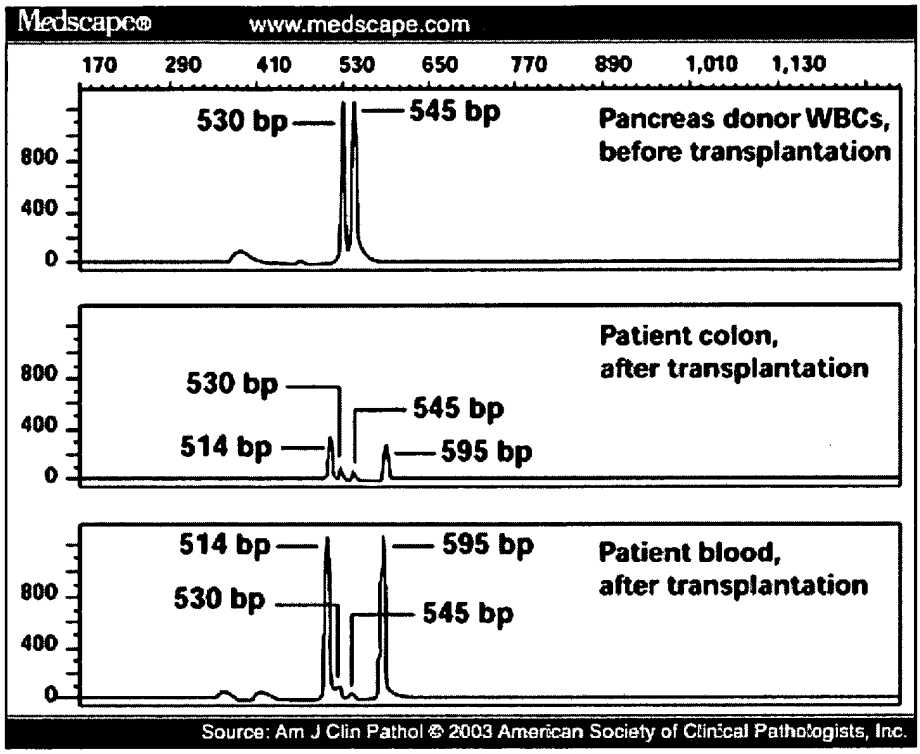


Figure 3. DNA from the pancreas donor for case 10 was heterozygous for the D1S80 marker with 2 distinct alleles of 530 base pairs (bp) and 545 bp. DNA from the recipient contained 4 distinct alleles (514, 530, 545, and 595 bp), consistent with chimerism; alleles of 514 and 595 bp are attributed to the patient. Of the DNA extracted from the colon biopsy specimen, approximately 80% was derived from the patient and 20% from the pancreas donor. The DNA extracted from the blood specimen also demonstrated the same chimeric pattern, with 94% of the DNA derived from the patient and 4% derived from the pancreas donor.

Two patients (cases 8 and 10) died of complications of bone marrow failure. In the other patients, symptoms resolved spontaneously or after immunosuppression was decreased.

Discussion

Transplanted solid organs contain a variable amount of lymphoid tissue, enabling the allografts to function as a mini bone marrow transplant.[14] Cell migration from the donor organ to lymphoid and nonlymphoid organs and chimerism are documented after solid organ transplantation in animals and in humans.[15] In these chimeric conditions, the outcome is determined by the balance between the recipient's immune system and the donor's WBCs.[14, 16] On one end is the case in which recipient cells prevail, resulting in rejection. The second possibility is the state of tolerance in which neither donor nor recipient cells prevail. In successful transplantations, using donor-specific HLA alleles as markers, chimerism was found in all 22 liver recipients who were studied 10 to 21 years after transplantation and in all 5 renal transplant recipients studied 27 to 29 years after transplantation.[14, 15, 17] Similar results were observed in recipients of thoracic organs.[18] It has been postulated that donor lymphoid cells are important in long-term graft survival. The third possible outcome is GVHD in which donor WBCs dominate. GVHD has been reported after almost all kinds of solid organ transplantation. Although GVHD is seen most frequently after liver transplantation,[2, 5, 19-22] the incidence of GVHD is highest after small bowel transplantation (about 5%), possibly owing to a large number of donor lymphocytes present in the transplanted organ.[16, 23]

During a 9-year period we identified 10 patients in whom GVHD developed after solid organ transplantation. Six patients had a histologic diagnosis of GVHD within 10 weeks of blood product transfusion and/or solid organ transplantation. Of those, 2 patients (cases 5 and 6) received a blood product transfusion or underwent solid organ transplantation within 3 months before the biopsies, which is consistent with blood product transfusion or solid organ transplantation being the likely source of alloreactive lymphocytes. In 4 patients (cases 1, 7, 8, and 10), blood product transfusion, solid organ transplantation, or both may be the source of alloreactive lymphocytes since both

were done within the 3-month period before the biopsies. In the 1 patient in whom the chimerism studies could be performed (case 10), 20% of the DNA extracted from the colon biopsy specimen and 4% of the DNA from the blood specimen originated from the pancreas donor. Chimerism based on HLA or DNA evidence has been reported in 24 of 43 liver, 2 of 4 kidney, 1 of 1 heart, and 2 of 4 pancreas-spleen-duodenum transplant-related cases of GVHD.[16] Most of the time the lymphocytes are from the solid organ donor. However, transfusion-derived lymphocytes were documented to be the cause of GVHD in a heart and a liver transplant recipient by HLA-DNA typing.[16]

Usually skin involvement precedes liver and gut involvement. Although the histologic findings of mild GVHD involving skin are not specific, in bone marrow transplant recipients, skin biopsies commonly are performed to diagnose GVHD and to rule out other conditions such as drug reactions. The absence of skin biopsy specimens for most of our cases probably is due to a lack of clinical suspicion of GVHD. An erythematous skin rash in solid organ transplant recipients frequently is attributed to drug reactions and not to GVHD. In addition, sometimes gastrointestinal tract GVHD may occur in the absence of skin involvement.[24] One patient (case 8) had severe involvement of the skin with bullae formation prompting a skin biopsy, which showed grade III GVHD.

Most patients in the present series had gastrointestinal tract symptoms. Gastrointestinal tract biopsies were done mainly to rule out an infectious process in these immunocompromised patients. The biopsies revealed a spectrum of findings associated with GVHD ranging from apoptotic cells in the intestinal epithelium in the absence of significant inflammation (grade I) to denudation of the mucosa with few residual remaining glands (grade IV).

Although no single specific histologic finding for GVHD exists, the gastrointestinal tract lesions are thought to be more specific and are characterized by epithelial cell injury: apoptosis, glandular destruction, and debris in the glands out of proportion to the density of inflammatory infiltrate in the lamina propria.[11] Glandular dilatation, intraepithelial lymphocytosis, and crypt abscess are nonspecific but commonly seen in GVHD of the gastrointestinal tract.[25] In 1 study, apoptosis was found to be the most sensitive marker of GVHD (sensitivity, 84%; specificity, 66%) compared with other findings.[24] However, GVHD-like lesions may be seen in patients with CMV and HIV infections, in patients with primary immunodeficiencies, and in patients who are taking nonsteroidal anti-inflammatory drugs or undergoing cytoreductive or radiation therapy.[24, 26]

Four patients (cases 2-4 and 9) underwent gastrointestinal tract biopsies 7 to 34 months after blood product transfusion and solid organ transplantation, much longer than the usual 1- to 11-week interval. Two of these 4 were taking the immunosuppressive drug mycophenolate mofetil (CellCept, Roche Laboratories, Nutley, NJ), which recently has been reported to cause colitis with histologic features mimicking GVHD.[26] In the patients in whom late GVHD-like lesions developed, the possibility of a mycophenolate mofetil-related colitis can not be eliminated, especially since 1 patient (case 9) recovered after discontinuation of mycophenolate mofetil. However, the other patient (case 3) had grade III GVHD of the colon, which is uncommonly severe for a drug-related reaction. It is conceivable that the immune dysregulation caused by this drug may facilitate the appearance of bona fide GVHD by hampering a functional T-cell response in the host that would favor the survival and proliferation of donor lymphocytes. Acute GVHD more than 3 months after blood product transfusion or solid organ transplantation has not been reported. Although this possibility cannot be ruled out entirely, in patients with a distant history of blood product transfusion and solid organ transplantation, other causes of GVHD-like reactions should be considered.

CMV colitis may simulate acute GVHD.[25, 27] In 9 cases, no viral cytopathic change was evident on histologic examination, and immunohistochemical stains were negative. One patient (case 10) had CMV in multiple gastrointestinal tract biopsy specimens. However, the extent of glandular destruction and apoptosis was disproportionate to the number of viral inclusions. Additional tissue from the gastrointestinal tract showed chimerism as described herein.

The exact incidence of GVHD after solid organ transplantation is not known. Mild cases probably remain undiagnosed because the clinical features of fever, rash, and diarrhea can be misinterpreted as being of infectious origin during the posttransplantation period. Since GVHD after solid organ transplantation is a potentially lethal disease and because the patients rarely would have a clinical diagnosis of GVHD, recognition of the histologic features is crucial for accurate diagnosis and treatment. Of 10 patients, 2 died of complications of bone marrow failure.

GVHD is not limited to the allogeneic bone marrow transplant recipients. GVHD can be seen after solid organ transplantation and in recipients of nonirradiated blood products secondary to alloreactive lymphocytes. No single specific clinical, endoscopic, or histopathologic finding exists for GVHD after solid organ transplantation. Histologic GVHD-like reactions may be seen in other conditions such as CMV colitis and drug reactions. A high index of

suspicion is necessary for the correct diagnosis because GVHD may cause bone marrow failure and death.[1, 9, 19, 28] Molecular studies to evaluate the presence of donor lymphocytes in the affected organ (chimerism) help support the diagnosis of GVHD in equivocal cases.

Tables

Table 1. Clinical Characteristics, Transplant Information, and Pathologic Findings

Case No/Age (y)	Transplant Type	Donor	No. of HLA Mismatches	Time to Biopsy From		Biopsy Site	Graft-vs-Host Disease Grade
				Transplant	Blood Product Transfusion		
1/57	Lung	Cadaver	6	9 wk	7 wk	Stomach	I
2/41	Kidney	Living related	3	9 mo	9 mo	Rectum	I
3/36	Kidney	Living related	3	17 mo	25 mo	Colon	III
4/44	Kidney	Living related	2	34 mo	7 mo	Duodenum	I
5/30	Kidney	Living related	3	68 mo	1 d	Colon	IV
						Small bowel	IV
						Stomach	IV
6/41	Kidney	Living related	4	6 d	5 mo	Stomach	I
7/49	Liver	Cadaver	5	8 d	8 d	Colon	IV
8/58	Pancreas	Cadaver	4	7 wk	5 d	Stomach	I
	Kidney					Skin	III
9/58	Heart	Cadaver	5	21 mo	22 mo	Rectum	II
10/32	Pancreas	Cadaver	3	11 wk	10 wk	Colon	III
	Kidney	Living related	5			Liver	I

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Clinical Transplantation

Graft-vs.-host disease in lung and other solid organ transplant recipients

Assi MA, Pulido JS, Peters SG, McCannel CA, Razonable RR. Graft-vs.-host disease in lung and other solid organ transplant recipients. Clin Transplant 2007: 21: 1–6. © Blackwell Munksgaard, 2006

Abstract: Graft-vs.-host disease (GVHD) is an uncommon complication of solid organ transplantation. Herein, we report a case of GVHD occurring in a lung transplant recipient and review 29 reported cases of GVHD that complicated thoracic organ, and non-hepatic intra-abdominal organ transplantation. The major presenting clinical symptom of GVHD was skin rash. Less frequent clinical manifestations were cytopenia (16%), diarrhea (11%), and fever (5%). The mainstay of treatment was high-dose corticosteroids. The mortality rate was high (30%). The cause of death was mainly due to infection, suggesting that antimicrobial prophylaxis may improve the outcome of this potentially fatal complication.

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Key words: chorioretinopathy – graft-vs.-host – graft-vs.-host disease – solid organ transplant

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Graft-vs.-host disease (GVHD), a common complication of allogeneic hematopoietic stem cell transplantation, occurs much less frequently after solid organ transplantation. GVHD has, however, been recognized as a devastating complication of liver transplantation with the first case being reported in 1988 (1). The scientific literature suggests that GVHD following solid organ transplantation is most commonly encountered in liver transplant recipients and is associated with very high mortality in this population. Data regarding GVHD in other (non-hepatic) solid organ transplant recipients are much more limited, hence the assumption that it occurs much less frequently in this patient population.

With the aim of characterizing the clinical features and outcomes of GVHD among recipients of solid allografts other than a solitary liver, we identified cases occurring in our institution and reviewed the scientific literature for all reported cases of GVHD in this patient population. Our review identified only a single case of GVHD in our institution occurring in a lung transplant recipient (presented in abstract form by McCannel CA, Seventeenth Annual Ocular Angiography Conference, August 3–6, 2005, Whistler, British Columbia, Canada). No cases were identified in kidney, pancreas, and heart transplant recipients.

Case report

A 25-yr-old male with primary pulmonary hypertension underwent bilateral lung transplantation on October 15, 2004. He received induction immunosuppressive therapy with OKT3, azathioprine, and dexamethasone. On day 17 after transplant, he presented with a generalized pruritic rash and blurry vision. At that time, he was receiving prednisone (10 mg bid), cyclosporine A (150 mg bid), and azathioprine (200 mg qd).

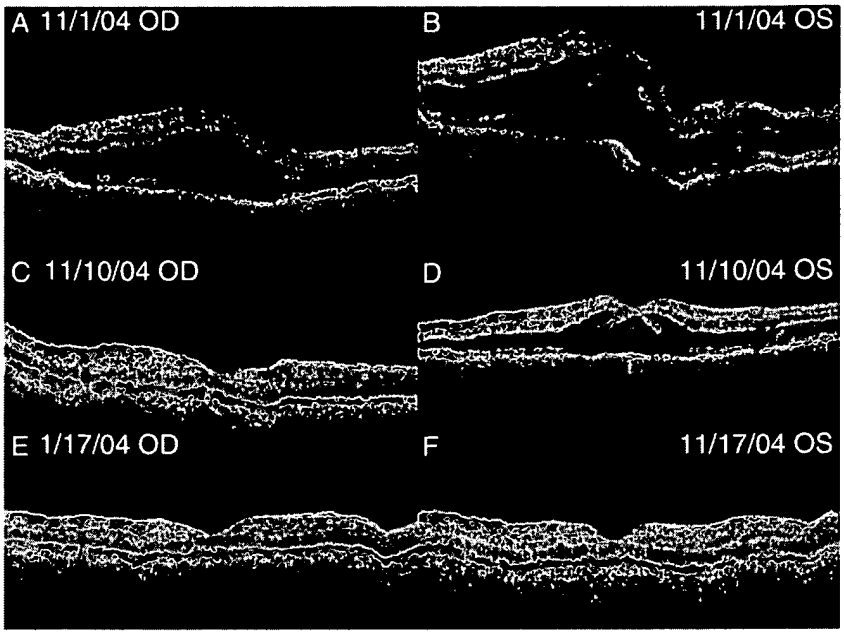

Fig. 1. Panels (A, B): retinal detachment in a patient with bilateral lung transplant and graft-vs.-host disease (right and left eye, respectively); panels (C, D): improvement in retinal detachment (right C better than left D) after 10 d of corticosteroids; panels (E, F): complete resolution of retinal detachment in both eyes after 17 d of corticosteroids.

A skin biopsy showed dermal inflammation and features suggestive of GVHD. Opthalmologic examination revealed serous chorioretinopathy and bilateral retinal detachment that was thought to be secondary to GVHD (Fig. 1). Prednisone was increased to 20 mg bid, which resulted in the resolution of his skin rash and ocular symptoms within three wk. There were no changes in his other immunosuppressive medications and antimicrobial prophylaxis. He remained on prednisone 20 mg bid for two wk and was tapered gradually over five wk to 8 mg bid. At the time of last follow up in 2006, he remained with good allograft function. He has not had any recurrence of his ocular and dermatologic symptoms.

Literature review

A Medline search was conducted to identify reported cases of GVHD in solid organ transplantation from January 1, 1960 to November 1, 2005 using the terms "GVHD" and "organ transplant." A secondary search was performed to include references cited in the articles obtained on the initial search. Including the patient we are reporting herein, there was a total of 30 cases of GVHD occurring in recipients of solid organs other than a solitary liver allograft (2–9). Eleven patients were recipients of small bowel or combined small bowel-liver allografts; nine were recipients of kidney, combined kidney–pancreas, and pancreas–spleen allografts, five were recipients of heart, lung, or combined heart–lung allografts, and five had multivisceral transplants. The median age of all patients was 32 yr (range, eight months to 58 yr). Twenty patients were adults and nine were children (no age was reported in one case). Most pediatric cases were females who had undergone small bowel transplantation.

The clinical characteristics of the cases are presented in Table 1. Of the 30 reported cases, the clinical manifestations of GVHD were reported only in 19 cases. The median time interval from the date of transplantation to the onset of clinical symptoms was 33 d, although it occurred later after kidney transplantation (median of 77 d). All 19 patients (100%) had a skin rash. Other less frequent manifestations were cytopenia (16%), diarrhea (11%), and fever (5%). No ocular manifestations were reported.

Donor cell chimerism was detected in 12 of 19 patients in which human leukocyte antigen (HLA) typing was performed (2, 3, 5–7, 9). One patient had donor DNA isolated from a skin biopsy by variable-number tandem repeat analysis (8). Fluorescent *in situ* hybridization (FISH) analysis identified the Y chromosome in a skin biopsy specimen of a female recipient who received the allograft from a male donor (4).

Treatment modalities were reported in detail only in five of the 30 patients. Four patients received corticosteroids, with two of these patients also receiving anti-lymphocyte antibodies. In the fifth patient, who is a recipient of a lung transplant, treatment was with mediastinal irradiation only (7). The overall mortality rate among the reported cases was 30% (nine of 30 cases); the mortality rate for pediatric cases was 22% compared with 33% in

Table 1. Characteristics of 30 patients who developed graft-vs.-host disease after solid organ transplantation

	Total	Pediatrics	Adults	Liver/SB, SB	K, K/P, P/S	H, L, H/L	MV
Cases	30[a]	9[a]	20[a]	11	9	5	5
Age in years (median)	32 (29)[b]	1.3	41	11.6	38	44 (4)	35
Sex (male)	45% (20)	30%	55% (11)	27%	100% (2)	50% (2)	60%
Median time to onset in days	33 (16)	12 (2)	56 (14)	12	77	17	NR
Fever	5% (19)	0%	8% (12)	0	33% (3)	0	0
Rash	100% (19)	100% (6)	100% (12)	100% (8)	100% (3)	100% (3)	100% (5)
Diarrhea	11% (19)	0%	8% (12)	0	0	33% (3)	0
Cytopenia	16% (19)	0%	25% (12)	0	66% (3)	33% (3)	0
Death	30% (30)	22% (9)	33% (21)	18% (11)	44% (9)	40% (5)	20% (5)
Median time to death in days	23 (5)[b]	23 (2)	32 (3)	23 (2)	41 (2)	32 (1)	NR

SB, small bowel; K, kidney; K/P, kidney and pancreas; P/S, pancreas and spleen; H, heart; L, lung; H/L, heart and lung; MV, multivisceral; NR, not reported.
[a]No age was reported in one case.
[b]Total number of patients for which information were available.

adults. The median interval time from clinical presentation to death was 23 d. The causes of death were reported in seven of nine patients, with most (71%) of these patients dying from infectious complications.

Discussion

This study reviewed the clinical features and outcomes of 30 cases of GVHD that occurred in recipients of solid organ allografts other than a single liver. This review demonstrates that GVHD, which presents most commonly as skin rash, is a very uncommon yet highly fatal complication of solid organ transplantation.

More than one-third of the reported cases were recipients of small bowel transplant, while only eight cases were recipients of kidney transplant. This ratio does not reflect the overall distribution of solid organ transplantation in the USA, as kidney transplant is much more commonly performed than small bowel transplant (16 477 vs. 178 transplants in 2005 according to the United Network of Organ Sharing database). Hence, while this disproportionate ratio of GVHD in small bowel and kidney transplantation may be the result of reporting bias, it is also very likely a reflection of the pathogenesis of GVHD in solid organ transplantation. Specifically, it is believed that GVHD in solid organ transplantation is related to the lymphoid characteristic of the transplanted organ – small bowel carrying much more lymphatic tissue than kidney. The highest number of solid organ transplant-related GVHD cases occurred in liver transplant population (2, 3, 10–49). This supports the theory that the pathogenesis of GVHD is dependent on the transfer of a high enough "inoculum" of lymphocytes, probably through lymphoid tissue within and surrounding the organ being transplanted (50). In this regard, recipients of lung allografts, which also carry high amount of lymphoid tissue, may also be at higher risk.

This review highlights the onset of GVHD at a relatively early period following solid organ transplantation, with a median of 33 d. However, kidney transplant recipients seem to present at a later date, with a case manifesting at nine months after transplant. Skin rash is the most common clinical manifestation, thereby making the definitive diagnosis of GVHD somewhat difficult. Transplant recipients receive a variety of medications, including sulfa and azole drugs for antimicrobial prophylaxis – drugs which are well known to cause dermatologic and allergic manifestations. A high index of suspicion is therefore needed to make a timely diagnosis. In this regard, there should be a low threshold for performing skin biopsy, which could show epidermal dyskeratosis and epithelial cell necrosis suggestive, albeit non-confirmatory, of GVHD (51). Other clinical findings like fever, diarrhea and cytopenias may also suggest another systemic illness, including those with an infectious etiology. More specific testing, like FISH analysis looking for donor-derived Y chromosomes in a female transplant recipient (39) and peripheral HLA typing (1) looking for macrochimerism (defined as $>1\%$ donor nucleated cells in the peripheral blood) are useful for making the GVHD diagnosis.

The lung transplant recipient seen at our institution developed chorioretinopathy and retinal detachment simultaneously with the skin rash suggestive of GVHD. The patient responded to a course of high-dose oral prednisone with the concomitant resolution of the skin and ophthalmologic symptoms. Several reports in the literature have described uveal effusions and retinal detachment similar to our patient's ocular findings in

association with pulmonary hypertension (52–54). Our patient had previously complained of blurry vision two months prior to transplantation and he was presumed to have ophthalmologic changes related to his pulmonary hypertension, although the definitive documentation of these findings is not available. Hence, while an alternative explanation for the retinal changes in our patient could be related to pulmonary hypertension, we would not have expected these to recur two wk after transplantation when the pulmonary pressure has normalized. Furthermore, the ocular findings in our patient resolved concomitantly with the improvement in skin rash following the intensification of immune suppression. Collectively, these suggest ocular GVHD as the underlying etiology of our patient's ocular pathology. Indeed, retinal manifestations of GVHD have been previously described in hematopoietic stem cell transplantation (55, 56). One patient developed central serous chorioretinopathy (CSCR) associated with GVHD after undergoing allogeneic bone marrow transplant (BMT) for acute myelogenous leukemia. While CSCR could be related to systemic corticosteroids and systemic hypertension, the role of GVHD as a contributing factor was not ruled out (55). Another patient, who developed acute GVHD involving the skin and gastrointestinal tract 11 d following allogeneic BMT for multiple myeloma (56), presented with decreased vision bilaterally at day 16 after transplantation. Funduscopic examination and fluorescein angiography were consistent with bilateral multifocal CSCR. After the initiation of intravenous methylprednisolone and cyclosporine A, the exudative detachments gradually resolved over the next three months. All these anecdotal reports, together with our case, indicate that GVHD may affect the choroidal vasculature, leading to choroidal hyperpermeability and the development of CSCR.

It is not clear from the available data on whether the outcome of GVHD in solid organ transplant recipients will be improved by earlier diagnosis and treatment. The mainstay of treatment has been corticosteroids. Discontinuing or decreasing the dose of other concomitant immunosuppressive medications is a reported strategy in the liver transplant literature (3). Anti-lymphocytes have also been used, mostly in severe cases of GVHD after liver transplantation, with varying results (1, 3, 5, 9–14, 16, 17, 27, 30–32, 34, 35, 39, 45, 46, 48, 49). Data on the utility of newer immunosuppressive medications like anti-interleukin 2 receptor antibodies are currently scarce (3, 10, 13, 36).

The mortality rate in the patient population we studied was 30%; this appears to be lower than that reported in liver transplant-associated GVHD (mortality rate of >60%). Infection and multiorgan failure are the most common reported causes of death in solid organ associated GVHD cases (2, 3, 6–11, 14, 30, 32–35, 39, 41–43, 46–48). Fungi and cytomegalovirus (CMV) are frequently identified as the etiologic agents (2, 7, 9, 11, 14, 22, 30, 34, 41, 42, 46, 48). Bacterial infections might be less common as these severely ill patients often receive empiric broad-spectrum antibiotics. As fungi and CMV are the most frequent infectious causes of death, the use of antifungal and anti-CMV (in seropositive or mismatched patients) prophylaxis is probably indicated and could improve overall outcome.

In conclusion, GVHD occurs rarely in recipients of solid allografts other than solitary liver. The clinical manifestations are generally non-specific but always included skin rash. The mainstay of treatment was high-dose corticosteroids. The mortality rate was high and was mainly due to infections, suggesting that antimicrobial prophylaxis may benefit the small number of patients that develop this highly fatal complication.

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Preventing graft-vs.-host disease after bone marrow transplant--without toxicity

Trial will test a natural antibiotic depleted by pre-transplant chemotherapy

Monday, December 11, 2006

Unless the donor is an identical twin, patients undergoing bone-marrow transplant (also known as hematopoietic stem cell transplant, or HSCT) must first receive powerful chemotherapy drugs to wipe out their immune system and prevent their bodies from rejecting the donated cells. Research from Children's Hospital Boston and the Dana-Farber Cancer Institute has helped show that this punishing regimen increases the risk of graft-versus-host disease (GVHD), in which the donor's cells mount an immune response against the patient. But the most recent findings also suggest that the risk for GVHD can be reduced--with virtually no toxicity--by replacing a natural antibiotic protein that is depleted when patients undergo chemotherapy.

Now, a multicenter study is about to test this idea in HSCT patients, using a manufactured form of BPI known as rBPI21 (XOMA Ltd.) Unlike other treatments to prevent GVHD, BPI does not suppress the immune system and has shown virtually no toxicity.

Researchers Ofer Levy, MD, PhD, of Children's Hospital Boston, and Eva Guinan, MD, of Children's Hospital Boston and Dana-Farber Cancer Institute, will present their most recent findings and discuss the new clinical trial on December 11 at the American Society of Hematology (ASH) Annual Meeting in Orlando, Fla. **(abstract # 2856).** The new trial is the culmination of over five years of collaborative research by Levy and Guinan in human patients. "Many basic and translational studies, including our own, have provided a strong rationale for a trial of BPI in patients undergoing hematopoietic stem cell transplants,"

Additional Resources

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says Levy. "Replenishing a natural host defense factor that is deficient due to chemotherapy makes theoretical and practical sense, and we hope that bringing our bench work to patients will reduce the complications they suffer."

GVHD occurs when immune cells from donor attack the recipient, and can lead to multiple organ failure and death. It strikes some 30-60 percent of transplant patients, depending on how closely matched the donor is, and is kept in check only by eliminating otherwise useful donor immune cells or by using powerful immune-suppressing drugs.

Studies in mice had shown that the chemotherapy regimens used in HSCT not only wipe out white blood cells (with the intended effect of suppressing the immune system), but also damage the intestinal lining. This breach of the lining allows endotoxin, which is produced by bacteria living in the intestines, to enter the bloodstream. The endotoxin, in turn, provokes an inflammatory response that mobilizes donor immune cells, helping to trigger GVHD.

Levy, in Children's Division of Infectious Diseases, had long been studying BPI, which naturally blocks and neutralizes endotoxin. (1) BPI is found in neutrophils, the very white blood cells that are virtually wiped out by pre-transplant chemotherapy. Studies in mice had shown that blocking endotoxin reduces the incidence of GVHD after chemotherapy and HSCT. (2)

Intrigued by these findings, Levy and Guinan began to study endotoxin and BPI in human patients undergoing HSCT with pre-transplant chemotherapy. In 2003 they showed, in a study of 57 children, that patients' blood endotoxin levels rise markedly within a week of the transplant. (3) And now, in a study of 30 patient:donor pairs to be presented at the ASH meeting, they show that patients undergoing HSCT also have a sharp drop in BPI levels -- just as their endotoxin levels are rising -- and that BPI deficiency is associated with a greater likelihood of GVHD.

"BPI is markedly deficient -- 100 to 1000-fold lower -- in our transplant patients," says Guinan, associate director of the Center for Clinical and Translational Research at Dana-Farber. "If we can replenish this host defense factor, we might be able to moderate the damaging effects of GVHD."

The multicenter clinical trial, expected to begin within the next few months, will test rBPI21 (opebacan, NEUPREX® [Nasdaq: XOMA]). rBPI21 has been in phase I, II, and III human trials, with evidence of benefit in children and adolescents with serious meningococcal infections, but has not yet been approved by the Food and Drug Administration.

Levy and Guinan will first conduct a small safety trial, gradually increasing the amount of BPI given and the duration of treatment. If BPI appears safe, they will quickly

mount a randomized, controlled trial in 30 to 40 patients who are undergoing HSCT for cancer or blood diseases. Children's/Dana-Farber will be the lead center, with four to five additional pediatric and adult sites at prominent medical centers around the country.

"Our ultimate goal is to reduce the downstream complications of stem-cell transplant," says Guinan. "BPI would make these transplants significantly less toxic."

The study presented at the ASH meeting was supported by XOMA and private donations to Guinan's laboratory.

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Children's Hospital Boston is home to the world's largest research enterprise based at a pediatric medical center, where its discoveries have benefited both children and adults since 1869. More than 500 scientists, including eight members of the National Academy of Sciences, 11 members of the Institute of Medicine and 10 members of the Howard Hughes Medical Institute comprise Children's research community. Founded as a 20-bed hospital for children, Children's Hospital Boston today is a 347-bed comprehensive center for pediatric and adolescent health care grounded in the values of excellence in patient care and sensitivity to the complex needs and diversity of children and families. Children's also is the primary pediatric teaching affiliate of Harvard Medical School. For more information about the hospital and its research visit:
www.childrenshospital.org/newsroom.

Dana-Farber Cancer Institute (www.dana-farber.org) is a principal teaching affiliate of the Harvard Medical School and is among the leading cancer research and care centers in the United States. It is a founding member of the Dana-Farber/Harvard Cancer Center (DF/HCC), designated a comprehensive cancer center by the National Cancer Institute.

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SPECIAL REPORT

Eleven million donors in Bone Marrow Donors Worldwide! Time for reassessment?

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On 16 November 2005, we celebrated the milestone when 10 million donors had been registered in Bone Marrow Donors Worldwide (BMDW). Since then another million donors have been added in little more than a year. It seems an appropriate time for reassessment and to ask whether we are on the right track or not. We will do so by discussing the strength, weaknesses, opportunities and threats of the unrelated stem cell donor operation.
Bone Marrow Transplantation (2008) **41,** 1–9;
doi:10.1038/sj.bmt.1705866; published online 5 November 2007
Keywords: unrelated stem cell donor selection; stem cell donor recruitment; Bone Marrow Donors Worldwide; World Marrow Donor Association; non-inherited maternal antigens; acceptable mismatches

A bit of history

It was in 1964, now over 40 years ago, that a patient's life was saved for the first time thanks to our—at that time rudimentary—knowledge of human leucocyte antigen (HLA). It concerned a 42-year-old, multiparous woman, suffering from a chloramphenicol-induced bone marrow aplasia, who was admitted to the Leiden University Hospital, because of life-threatening bleeding from all orifices. She was initially treated with platelet concentrates from randomly selected blood donors, at that time a brand new form of treatment. These transfusions were able to stop bleeding for about a month, but then leucocyte antibodies appeared in her serum impairing platelet recovery after transfusion to near zero and she started to bleed again. Fortunately, she had a large number of siblings from which HLA compatible donors could be selected. Their platelets had again a good recovery and after 3 months of HLA-matched platelet support the bone marrow aplasia disappeared and she made a full recovery.[1]

In 1968, our group in Leiden and Robert Good's group in Minneapolis knew enough about HLA matching to make it possible for the first time to select HLA-identical sibling bone marrow donors for three patients suffering from congenital immune deficiencies. All three patients were cured by their sibling donor transplants.[2] It was soon realised that only one out of three patients had an HLA-identical sibling donor, and in 1970 it was proposed to start an European file of HLA typed blood transfusion donors, named Europdonor, to help patients in need of HLA-matched platelets or bone marrow, but without an HLA-identical sibling donor. The idea did not catch on in the other European countries at that time, but Europdonor has been functioning in The Netherlands since 1970, mainly by providing HLA-matched platelets, and occasionally HLA-matched unrelated bone marrow.[3–5]

In 1974, Shirley Nolan started what is now the Anthony Nolan Trust to find a donor for her son suffering from Wiskott–Aldrich syndrome. 'The Nolan' became the first registry of unrelated bone marrow donors routinely providing bone marrow first in the UK and soon all over the world.[6] In the 1980s, other European countries and the USA followed this example. By 1988, there were eight active registries with together 150 000 donors. Finding a suitable donor in the pre-Internet time was cumbersome and time-consuming and the proposal to start a listing of the HLA phenotypes, Bone Marrow Donors Worldwide (BMDW), was soon accepted.[7] In 1993, Pablo Rubinstein started collections of cord blood units in the first Cord Blood Bank at the New York Blood Center (NYBC) and issued the grafts for the first two matched, unrelated cord blood transplants.[8]

The number of registries and donors continued to grow. Today over 11 million bone marrow donors and cord blood units from 58 registries and 36 cord blood banks are available to provide life-saving stem cells to patients in need of them and can easily be contacted through BMDW.[9]

Strength

There is no doubt that our strength lies with these millions of donors: they are a symbol of hope and testamonial to the

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goodwill to people in the world united in their effort to provide life-saving help to those who need it. That is our strength. Without these donors we, the registries and cord blood banks, would not exist.

Every country celebrated 16 November 2005 in its own way. In France, donors from different countries discussed how they felt about being a donor, in the USA donors and recipients met, and in Cyprus the prime minister proclaimed that the 10 millionth donor came from Cyprus, and why not?

Since 1997, the World Marrow Donor Association (WMDA) has made annual reports recording the number of unrelated stem cell donations, which makes quite interesting reading. Figure 1 shows that since 1997, stem cell donations have tripled, peripheral blood stem cell donations have become increasingly popular and that since 2003 cord blood transplants have doubled.[10] The fact that the number of transplants tripled implies that many patients—today qualifying for a stem cell transplant—did not get one in previous years. Of course indications for transplantation have increased significantly, but it remains an open question whether we are now providing a transplant to every patient in need of one. We will return to this point later on.

Fortunately the great majority of these stem cell donations occur without complications but the WMDA Clinical Working Group recorded 15 reports of serious side effects out of over 13 000 bone marrow donations and 22 out of 9000 after peripheral blood stem cell (PBSC) donations carried out between 2002 and 2005. Overall, they occur in a frequency of over 1 out of 600 donations (Table 1) Serious Events and Adverse Effects Registry (SEAR).

Weaknesses

The large number of donors is our strength and we stand in debt to them, but what are our weaknesses? Our prime and most serious weakness is that in the period 2000–2006, out of about 151 000 patients qualifying for an HLA unrelated donor (UD) transplant, only 64 720 received one. Figure 2 shows not only the transplants realised, the light grey bars, but also the number of patients looking for a donor.[10] Overall there is almost a three-fold difference. Clearly we must try to do better. Fortunately many of these patients had other options, such as an autologous or haploidentical transplant, but that does not take away the fact that they were opting for an UD and this did not materialise.

Why do almost two thirds of the patients not reach transplantation? Many causative factors can be identified.

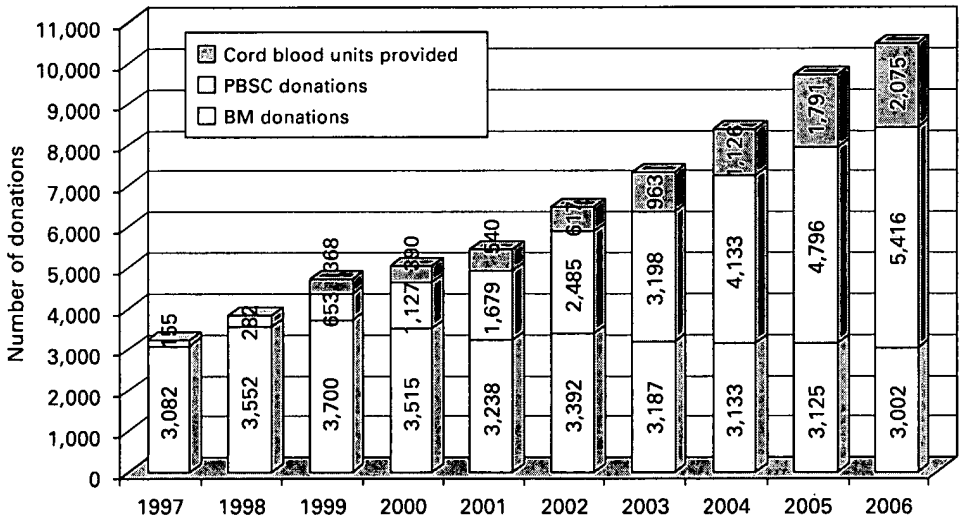

Figure 1 Since 1997 the WMDA office has collected the stem cell donations worldwide. Note that the peripheral blood stem cell (PBSC) donations doubled in the last 5 years, while the cord blood transplants doubled in the last 3 years.

Table 1 SEAR reports 2002–2005

15 reports after 13 331 BM donations
Atrium fibrillation, broken trocar in chest, laryngeal spasm, deep venous thrombosis, persistent lower back pain
22 reports after 9133 PBSC donations
Asystole during LP, cardiogenic syncope, gout attack, hospitalization (fever, emesis, headaches), severe bone pain, tetany, bleeding after removal of catheter, multiple infections

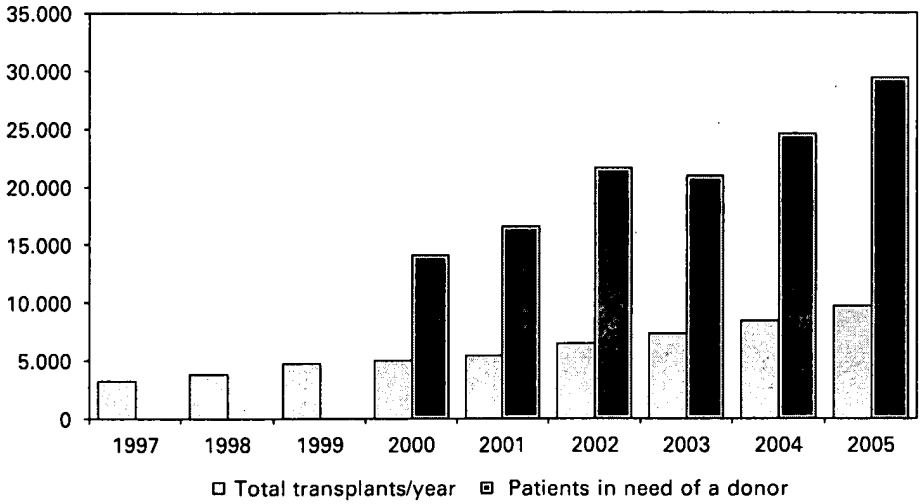

Figure 2 The number of patients in need of a donor has been calculated by counting the number of national patients opting for a donor as reported by the Hubs in the World Marrow Donor Association (WMDA) Annual Reports 2000–2006.

In the first place, the search process is overall much too slow. If we take Europdonor as an example, Heemskerk *et al.*[11] showed that for 30% of the patients, our search process and work-up for transplantation takes so much time, that when finally a donor has been located and found to be fit for donation, the patient has relapsed or is otherwise unfit for transplantation. As the average search time of Europdonor and patient work-up time in the Dutch transplant centres (TCs) is about the same as other registries, who have reported on this, there is *a priori* no reason to assume that the situation outside Europdonor is much different and very few registries provide blood samples for confirmatory high resolution typing within 15 working days, as recommended by WMDA.[12]

The enormous polymorphism of HLA is a second reason why only one out of three patients reaches transplantation. Even with our 11 million donors, only two out of three Caucasoid patients will find a 10 out of 10 HLA allele-matched donor; and for other racial groups, it can be as low as one out of four.[13] Unfortunately, there is still a sizeable number of TCs that insist on the availability of a 10 out of 10 HLA-matched donor before considering proceeding to transplantation. Of course, every transplant centre is and should be free to make its own rules, but one should realise that a graft with one or even more allele Class I mismatches still can lead to a survival at 9 years post-transplant of 25%, which compares favourably with death, a definitive certainty without alternative therapy[14,15] (Figure 3).

The transplant results are improving over the years, but it does so much more slowly than what has been achieved in organ transplantation. We should also remember that we have to try to match for all loci thus not only for HLA-A, -B and -DRB1, but also HLA-C, and perhaps -DQ and -DP, which now also are believed to influence graft prognosis.[14–16] That is certainly not all. A milestone paper by Kawase *et al.*[17] reporting on a study of over 5000 HSCT's performed in Japan show that certain amino acid substitutions influence stem cell transplant outcome unfavourably. Apart from the importance of incompatibilities

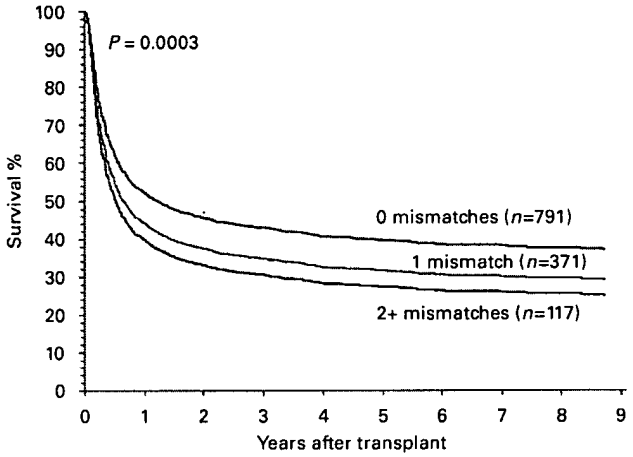

Figure 3 Risk-adjusted survival among HLA-A, -B serologic and -DRB1 allele-matched pairs by number of mismatched class I loci. Survival after transplantation was analysed as a function of the number of class I mismatches detected by high-resolution HLA typing in HLA-A, -B low resolution and -DRB1 high-resolution matched donor–recipient pairs. The data presented are adjusted for competing risk factors using the proportional hazards model, rather than univariate analysis. One or more additional mismatches led to poorer risk-adjusted survival ($P=0.0003$) (Flomenberg *et al.*).[15]

at certain amino acid positions,[17–19] additional loci in and outside HLA and KIR haplotype matching influence stem cell transplant outcome.[20–22] We certainly need a major effort to match for all these loci.

In the third place, the cost of finding an unrelated donor (UD) and obtaining the stem cells can preclude transplantation. It starts with the confirmatory typing (CT) samples, next when a donor is found the harvest costs, which vary from 12 000 to 25 000 Euro, with the cord bloods from 9000 to over 25 000 Euro and finally the transportation costs. The total costs can vary between 13 000 and 30 000 Euro; for quite a few patients or their insurance company this is a 'bridge too far'. These are estimates. The real costs in the different countries are largely unknown,[23] but they form a barrier to transplantation for many

patients. By identifying the real cost elements we might be able to reduce them.

One final and serious weakness is that the BMDW search operation has become outdated. When we started in 1988, there were eight registries with together in total no more than 150 000 donors. There are now 11 million donors and cord blood units in nearly 100 registries and cord blood banks. For about half of our Caucasoid patients, 20 or more HLA-A, -B and -DR split level identical donors spread out in these organisations are available. And one quarter of our patients have 100 or more split level matched HLA-identical donors! (H.G.M. van der Zanden, personal communication). Selecting a young (male) donor with the right cytomegalovirus (CMV) status for a given patient can have a major and favourable impact on transplant outcome.[24,25] How can one select quickly the most appropriate donor for a given patient without having information on age, gender, CMV status upfront in BMDW? We are in fact back to where we were in 1988! Unfortunately the Editorial Board (EB) of BMDW has as yet not been able to work out how this information can be implemented in BMDW without jeopardising donor confidentiality and registries logistics. The EB has here an important and urgent task to resolve.

Opportunities

How can we improve our operation? Fortunately, we have many opportunities to improve access and results and to reduce the costs. *Registries, search co-ordinators* and *TCs* each can make an important contribution to this end.

A logical and important first question to be asked by the *registries* is—considering the fact, that even Caucasoid patients have only a 60% chance of finding a 10 out of 10 allele HLA-matched donor—whether we need more donors? The answer is 'yes and no'. Of course, of young, preferably male donors, one never has enough! They should be targeted as replacement of the donors who retire because of old age or for other reasons. The registries can make an important contribution to speeding up the search process by typing *up-front* newly recruited donors at high resolution for HLA-A, -B, -C and -DRB1.

Because of different logistic reasons almost 20% of the registries have typed a large part of their donors for HLA-A and -B only. There are in total three million of such donors in BMDW, who are almost never used. Such registries can speed up the search process further by contacting those of these donors, who have a relatively low frequency HLA-A and -B phenotype and are not yet too old. If they confirm their willingness to donate stem cells, they could be retyped at high resolution for at least HLA-A, -B, -C and -DRB1.

As far as increasing the number of available phenotypes is concerned, increasing the number of donors is not cost-effective anymore if one recruits donors from a population with a NW European background. The frequent phenotypes are all there and in abundance and the so-called unique phenotypes, which one lacks, have a frequency of less than one in 11 million donors.[26] For populations with a different racial background than the NW European one,

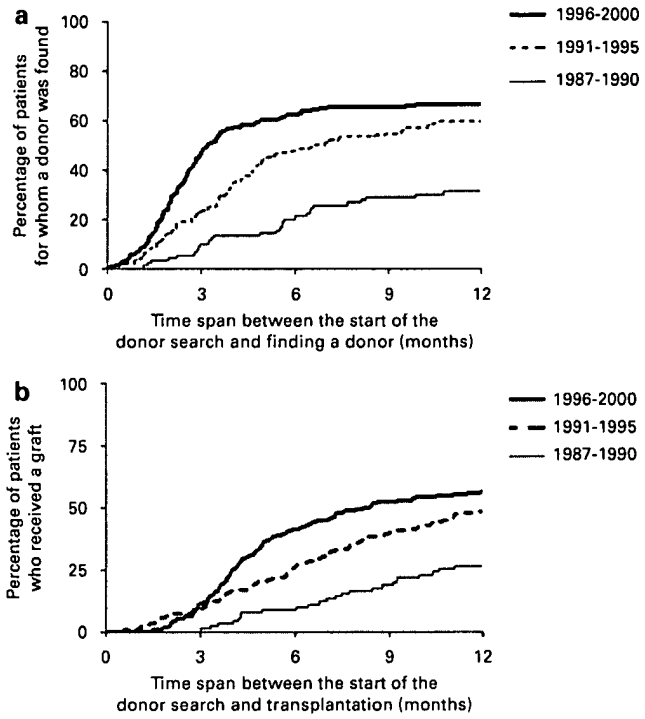

Figure 4 (a) Cumulative incidence of patients of northwest European origin who found a stem cell donor. The cumulative incidence increased per period (hazard ratio = 1.7; $P<0.001$) (Heemskerk *et al.*).[11] (b) Cumulative incidence of patients of northwest European origin who received a graft in 1 year after search start. The cumulative incidence increased per period (hazard ratio = 1.5; $P<0.001$) (Heemskerk *et al.*).[11]

we certainly need more donors, but these can for logistic reasons most efficiently be recruited via cord blood collection.[8]

As we just discussed, one of our major weaknesses is that it takes too long before a donor is selected, tested and the pre-transplant work-up of the patient is completed. Figure 4a shows the ED search time span data.[11] There is certainly an improvement during the last decade in the search time to locate a donor. However, after a donor has been selected, it takes also in The Netherlands an additional 2 months before the patient gets transplanted (Figure 4b). It must be possible to reduce the overall time quite substantially with no more than some clear guidelines between registries and TCs.

There is of course also a contradiction in Figures 4a and b. We and others have proudly published case histories, in which—because for example of accidental non-availability of bone marrow, while the patient had already been conditioned with a lethal dose of irradiation—we found, tested and collected the bone marrow from another donor within 7 days or even less![27] (S Cleaver, personal communication). The authors are birds of many feathers and we have in our background a blood banker's career (and blood banks run 7 days a week at 24 h service) and organ transplantation (and if a multi-organ donor becomes available, the heart and the liver and sometimes the kidneys are transplanted within 12 h) and we are taking the liberty of wondering whether we—in the registry world—should not rethink our logistics of the donor search and routinely

try to locate the best donor within weeks instead of months. As a consequence, the costs may rise further, but it would save lives.[11] This makes of course no sense if the TC cannot speed up their part of the operation as well.

These are easy, general statements and it will take quite some time before they can be implemented. However, there are quite a number of actions we can activate right now. In the first place, the *transplant co-ordinators* can make an important contribution by using BMDW up front for each and every search. It is done already by most search co-ordinators, but unfortunately not by all. If it is clear that a 10 out of 10 HLA-identical donor is not available, then accept that fact, do not lose time by looking for something which you know is not available, and go for an alternative. This can be a one or two allele-mismatched donor, a cord blood or a haploidentical donor.

If you select an allele-mismatched donor, you have the option to try to find out one with a negative cytotoxic T-lymphocyte precursor (CTLp) test. It has been shown that the CTLp tests, although difficult to perform and expensive, can discriminate between harmful and acceptable mismatches as illustrated in Figure 5.[19] A total of 53 patients with a single class I mismatched donor could be divided in a group with a negative CTLp test and a survival of over 60% at 7 years post-transplant, while survival was only 20% when the CTLp test was positive.[19]

Another way to effectively prevent delay before the transplant is the back-up donor. Van Walraven *et al.*[28] have shown that if a back-up donor is available, delay to transplant is only 7 days if the first choice donor is unable to donate, while without a back-up donor it is on average 79 days (Table 2). Note that 11% of the first choice donors can in the end not donate!

If a patient needs a transplant urgently and/or no 10 out of 10 donor is available, cord bloods can be very helpful and most importantly can save time. The two recent papers from the Center for International Blood and Marrow Transplant Research (CIBMTR)/New York Blood Center (NYBC) and EuroCord in the New England Journal of Medicine both made it clear that cord blood transplants even with one or more HLA-A, -B, -DRB1 mismatches (note: at the antigen level for -A and -B and only at allele level for HLA-DRB1) can give an adult patient a survival expectancy which is really not much worse than that of matched bone marrow donors[29,30] (Figure 6). As reported by Brunstein *et al.*[31] double cord blood transplants might be even able to improve on this. Many Cord Blood Banks have followed the example of the National Cord Blood Program of the NYBC (NCBP-NYBC) and make a special effort to store cord bloods from minority group donors, which are underrepresented in the registries.[8] This can have a major impact on the ability to provide stem cell transplants to patients from minority groups as illustrated in Table 3. In this table, the performances in the year 2002

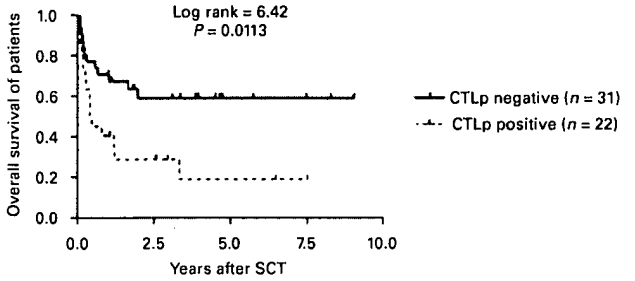

Figure 5 Patient survival of class I mismatched transplants improves significantly, if the CTLp test is negative (Heemskerk *et al.*).[19]

Table 2 The advantage in time of an initially identified back-up donor (the delay is defined as the difference between tentative and final harvest date)

	Median delay (days)	*Range (days)*	*% Transplanted*
Best donor available ($n = 371$)	0	0–155	87
Best donor deferred: back-up donor available ($n = 36$)	7	1–100	63
Best donor deferred: no back-up donor available ($n = 10$)	129	40–555	60

(Van Walraven *et al.*, 2005).[28]

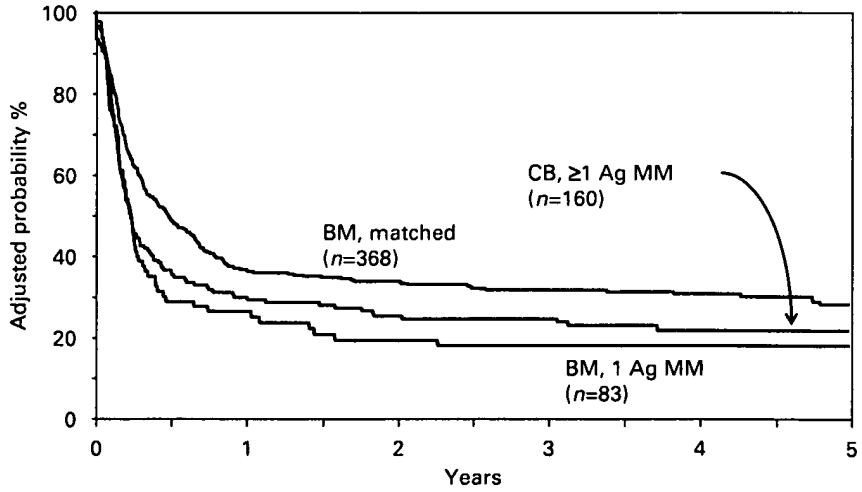

Figure 6 Adjusted probability of leukaemia-free and overall survival after bone marrow and cord blood transplantation (Laughlin *et al.*).[29]

Table 3 Bone marrow transplantation: ethnicity and access to transplants

	**NMDP data, GAO Report, 2002 '...equal access to a match may not be attainable'*		***NYBC research data 'Equal access to a match is attainable'*			
Ethnicity	*Searches*		*Donors*		*Transplants*	
	NMDP (%)	*NYBC (%)*	*NMDP (%)*	*NYBC (%)*	*NMDP (%)*	*NYBC (%)*
Asian	3.7	3.6	8	6.7	2.4	4.0
African American	12.1	12.1	10	24.6	6.3	19.0
Hispanic	12.5	14.0	11	23.5	7.8	15.7
Caucasian	69.1	67.8	67	45.2	81.9	58.5

Abbreviations: NMDP = National Marrow Donor Program; NYBC = New York Blood Center.
(P Rubinstein, personal communication).

of the NYBC and the National Marrow Donor Program (NMDP) are compared for their ability to provide stem cells to minority group patients. It is clear that the NYBC with, at that time, less than 40 000 cord blood units in stock, could provide grafts to a larger percentage of patients from minority groups than the NMDP with over four million donors. This is due not only to the advantage of cord blood HLA matching (for A and B at the antigen level and only for DRB1 at the allele level), but also reflects the relatively high numbers of unique HLA phenotypes present in cord blood banks as compared to registry donors.[8,9] The NMDP has since recruited over 50 000 cord blood units in order to be better able to provide stem cells to minority group patients.[1]

Many TCs follow nowadays a three line approach, if a patient is in urgent need of a transplant: not only is a suitable stem cell donor and/or cord blood looked for in BMDW, but also at the same time it is checked whether a suitable haploidentical family donor might be preferable. We will restrict our discussion on this issue to the haploidentical transplant with T-cell replete stem cells. This is possible because there is strong evidence that during pregnancy, a two-way exchange of cells between mother and child and vice versa takes place, which results in a sizeable likelihood of both mother and child acquiring a life-long partial tolerance to the other.

And this opens the possibility to perform T-cell replete stem cell transplants from mother to child, child to mother and between haploidentical sibling-to-sibling using standard conditioning and graft-versus-host disease (GVHD) prophylaxis.

A new HLA haplotype nomenclature is helpful in discussing these data (Figure 7). The child has inherited the maternal and paternal antigens from the parents respectively the IMAs and IPAs. It has not inherited the non-inherited maternal antigens (NIMAs), but it has been (through chimaerism) in contact with these antigens. Likewise, the mother has been in contact with IPAs of the child. If the mother donates to the child the GVH reaction will be directed to the IPA, when the child donates to the mother the GVH reaction will be directed to the NIMA. Likewise, haploidentical sibling transplants will only be done when the mismatch is NIMA-directed.

T-cell replete, one or two loci-mismatched haploidentical transplants between NIMA-mismatched siblings had less acute and chronic GVHD than a transplant from a non-

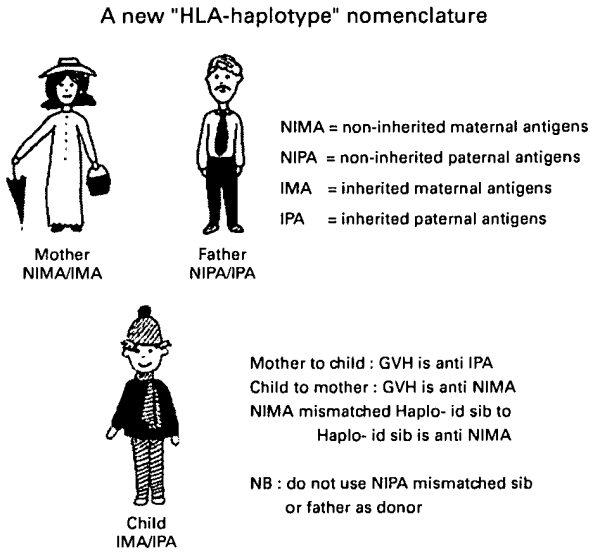

Figure 7 (See text for explanation).

inherited paternal antigens (NIPA)-mismatched sibling-to-sibling transplant or mother or father to child transplant. Although the GVHD is less in NIMA-mismatched sibling-to-sibling transplants, it is still increased as compared to HLA-identical sibling transplants (Figure 8).[32] Especially, in Japan, the experience with transplants using this alternative group of donors is now accumulating.[33,34]

The results with the mother as donor or as recipient (a new feature) are especially interesting. Transplants from mother to child, that is with an IPA mismatch, give more severe GVHD than child to mother or sibling-to-sibling NIMA-mismatched grafts. But for the whole group, if mutual chimaerism is present, overall survival after 2 years for those Japanese patients who were transplanted in remission was 60%, while for those who were refractory to therapy or in relapse survival was still 20% after 2 years (Figure 9).[33,34]

Threats

The short-term future of the donor registries and cord blood banks is not at risk. They fulfil a need and complement each other. This does not mean however that

we can sit back and relax. As discussed earlier and below, there are many aspects of the operation, which need our attention and can be improved. The difficulty is that so many independent organisations are involved. The WMDA has done and is doing a wonderful job as far as accreditation, regulations and ethics guidelines are concerned. The main difficulty lies in the fact, that there are so many independent registries and cord blood banks. Priorities of very large registries are quite different from those of the smaller ones, often working with one or a few TCs. However, even the largest registries are dependent on the 'import' of donors sometimes from quite small registries for a sizeable fraction of their national patients. In other words, there is a need for both the small and large registries.

We have discussed above the importance of selecting a donor with the right gender for a given patient, as young as possible and with the right CMV status. This is at the moment almost impossible to achieve, especially if 20 donors, in many different registries, are available, which is the case for half of our patients. The situation is further complicated by the fact that virtually all registries are dependent on their quantitative performance, that is the number of stem cell donations they realise to keep their budgets solvent. This can create conflict of interest, certainly when the operation is small and the co-ordinators of registry and transplant centre are one and the same person or two very closely collaborating individuals. As long as all donors in BMDW do not show all the above-mentioned information and/or are not typed for 10 loci at high resolution, it will be next to impossible to establish whether you have selected the best donor or not.

Since 1997, WMDA has been able to keep an almost complete track of unrelated stem cell and cord blood donations worldwide, but what happened to the patients who received these stem cells is only known in part. Some estimate the fraction of patients transplanted with an HSCT and with an adequate follow-up as in the order of less than 70% and then often with incomplete and near useless information as far as HLA is concerned (M Horowitz, personal communication). This is shameful and unacceptable for a therapy that is not only quite expensive and only partially successful, but also not without risk to the volunteer donors. WMDA, European Group for Blood and Marrow Transplantation (EBMT) and CIBMTR must all make a concerted effort to improve logistics and the reporting of transplant outcome. The transplant centre co-ordinators can contribute to this substantially by providing high-resolution typing data of the donor and the patient as well as age, gender and CMV status soon to be complemented by information on minor histocompatibility antigens and killer cell immunoglobulin-like receptor (KIR) typing. In this context, it might be worthwhile to study the possibility to use the software of the European Marrow Donor Information System (EMDIS), which has facilities to store the final HLA typing data of both patient and donor. An online transfer of these data to the EBMT and CIBMTR files would assure an easy and reliable sharing of information which is crucial for follow-up studies.

Another aspect of the operation, which is rarely discussed, concerns the question: 'Who is here in control'? One can question whether the continuous growth of millions of donors with a total cost of already now well over 3 billion Euro is really a good thing or are there

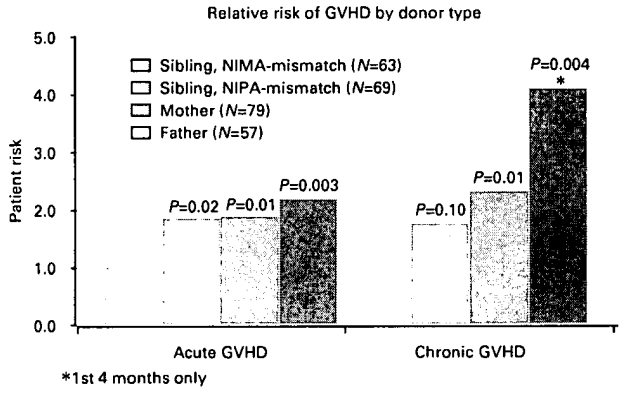

Figure 8 Maternal and paternal transplantations were associated with significantly more acute and chronic graft-versus-host disease (GVHD) and TRM than non-inherited maternal antigens (NIMA)-mismatched but not non-inherited paternal antigens (NIPA)-mismatched sibling transplantations. Graft failure and survival did not differ by donor type.[32]

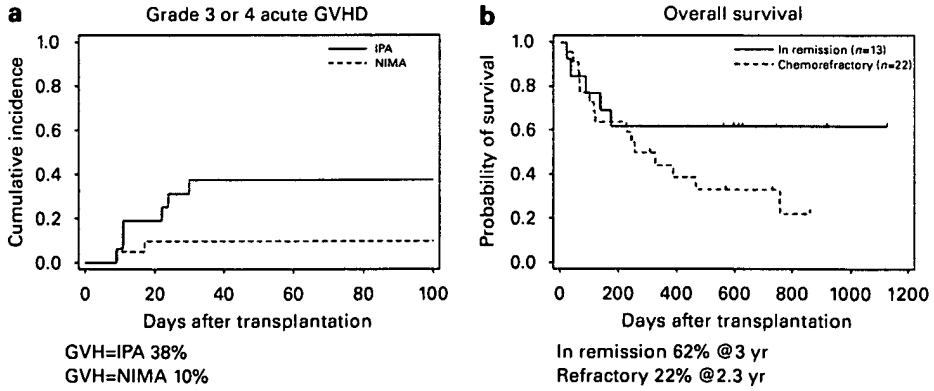

Figure 9 Results of a nation wide cross-sectional study of haploidentical IPA- or non-inherited maternal antigens (NIMA)-mismatched hemopoietic stem cell transplants. Adapted from Ichinohe *et al.*[33]

different or additional scenarios preferable? The Deutsche Knochen Mark Stiftung (DKMS) gives here a good example. They are not only successful in having new donors contributing to their own registration and HLA typing costs (which contributes to donor commitment), but they also type at high resolution young, male donors up front.[35] Unfortunately, they are the exception. In many registries, the comparison with the 'Sorcerer's Apprentice' opera is valid: donor recruitment is decentralised, often emotionally based and many of the recruited donors withdraw when actually confronted with a donation request. WMDA has in this context another educational task to deal with.

Looking at the annual WMDA reports, it is clear that we are witnessing a shift from the use of bone marrow donors to cord blood. At the moment cord blood's, major handicap is that if the patient has relapsed donor lymphocyte infusions are not possible. At least not yet! Protocols are in progress to resolve this, but that may take time.

There is another trend which is complicating the picture further, that is the storage of cord blood for autologous use. Started as a deplorable, money-making business, it has caught the public's eye and at this moment the number of cord blood units in storage for autologous use is two- to three-fold that of the public banks. And it is steadily increasing.

Autologous cord blood has been used occasionally, although the chance that the donor ever needs his or her own cord blood for a valid medical reason is small and probably less than 1 in 10 000. In Spain, autologous cord blood storage was forbidden by law, but this has been recently changed (M Fernandez, personal communication). It is now allowed under the following conditions:

1. The storage should be done following state-of-the-art guidelines.
2. HLA typing should be performed and reported to a certified public bank.
3. If a patient is in need of such an autologous stored cord blood (s)he has priority over the donor. In that case, the donor's family is reimbursed for the costs.

These are all very recent and interesting developments, which, in the end, result in the transfer of the cost of public banking to over-anxious parents of new and future babies. Time is required to conclude whether they are an improvement or not. Whatever the case, it is clear that such a strategy should be based on globally accepted ethics and regulation.

Concluding remarks

If we look at what has been achieved in the last 40 years in stem cell transplantation, it is clear that enormous progress has been made. It is equally clear that stem cell transplantation is still a half way technique, since it is not yet available to the majority of patients in need and it needs further improvement, or replacement (such as has been achieved by imatinib for chronic myeloid leukaemia). One of the major obstacles to further progress is that the number of transplants annually performed is relatively small, particularly, if we take into account the heterogeneity of the patient population as far as diagnosis, stage of disease and age are concerned. The situation is further complicated by the fact that these transplants are performed in hundreds of autonomous TCs. They collaborate in case-controlled studies as well in an increasing number of randomised prospective trials, but these rarely address major issues such as a randomised prospective trial of bone marrow versus PBSC versus cord blood transplantation with one or two donors. Another weak point is that most follow-up studies start at the moment when an intention to treat, that is stem cell transplantation has been decided upon, while it is more relevant to start a follow-up study of the therapy of a disease at the time of diagnosis.

Patients and their families have influenced the development of stem cell transplantation in a strong and positive manner beginning with Shirley Nolan and followed by Fred Hutchinson, Jose Carreras, Peter Harf and many others. There is no other form of treatment in which the family is so directly and physically involved as stem cell transplantation. The Internet has made family members well informed and often solid partners in our efforts to improve our results. They deserve to be involved in our planning for the future and decision making.

Acknowledgements

This review should be considered as a tribute to Shirley Nolan, a most remarkable woman with a vision, who remains an example and a challenge to all of us. The authors would like to thank the staff of Europdonor, as well as Profs A Brand and FHJ Claas, P Rubinstein, Tatsuo Ichinohe and Susan Cleaver for their input and positive and critical assessment of this review.

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